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Lowering of Serum Thyroxine and Triiodothyronine Levels in Yearling Coho Salmon, *Oncorhynchus kisutch*, by Dietary Mirex and PCBs

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LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1978. Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon, *Oncorhynchus kisutch*, by dietary Mirex and PCBs. J. Fish. Res. Board Can. 35: 1285–1289.

Yearling coho salmon, *Oncorhynchus kisutch*, were fed diets contaminated with PCBs (500 μg or 50 μg/g dry feed), Mirex (50 μg or 5 μg/g dry feed), or a mixture of Mirex and PCBs (5 μg Mirex + 50 μg PCB/g dry feed) daily for 2 or 3 mo. Serum T3 and T4 levels were significantly reduced in fish fed the high Mirex diet for 2 or 3 mo. Serum T3 levels were reduced in fish fed the high PCB diet for 3 mo. Serum T3 and T4 levels were significantly reduced in fish fed the mixed Mirex–PCB diet for 3 mo. The significant increase in serum T4/T3 ratios in fish given the high Mirex or high PCB diet was not evident in fish given the mixed Mirex–PCB diet, suggesting a synergistic effect that is different from the effect of individual contaminants. Fish given the high PCB or high Mirex diet had significantly lower body weights than the control fish.

Key words: organochlorines, teleost fish, thyroid gland, hormones, endocrines, synergism, pituitary gland

LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1978. Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon, *Oncorhynchus kisutch*, by dietary Mirex and PCBs. J. Fish. Res. Board Can. 35: 1285–1289.

Nous avons donné à des saumons coho (*Oncorhynchus kisutch*) d'un an une nourriture contaminée par des BPC (500 μg ou 50 μg/g de nourriture sèche), du Mirex (50 μg ou 5 μg/g de nourriture sèche) et par un mélange de Mirex et de BPC (5 μg de Mirex + 50 μg BPC/g de nourriture sèche) quotidiennement pendant 2 ou 3 mo. Les niveaux de T3 et T4 sériques sont nettement diminués chez les poissons nourris au régime contenant une forte concentration de Mirex pendant 2 ou 3 mo. Les niveaux de T3 sérique sont

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diminués chez les poissons nourris au régime de forte teneur en BPC pendant 3 mo. Les niveaux de T3 et T4 sériques sont nettement diminués chez les poissons nourris à un mélange Mirex–BPC pendant 3 mo. Les poissons nourris au mélange Mirex–BPC ne montrent pas d'augmentation significative des rapports T4/T3 sériques observés chez les poissons nourris aux régimes riches en Mirex ou en BPC, ce qui suggère un effet de synergie différent de l'effet de contaminants pris individuellement. Les poissons nourris aux régimes à forte teneur en BPC ou en Mirex ont des poids corporels inférieurs à ceux des poissons témoins.

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Coho salmon populations in the Great Lakes show evidence of goiter formation and hypothyroidism (Robertson and Chaney 1953; Black and Simpson 1974; Drongowski et al. 1975; Sonstegard and Leatherland 1976; Moccia et al. 1977). Epizootiological studies of goiter frequency indicate that although low lake iodide levels undoubtedly contribute to the development of goiters in these populations, environmental factors may also be involved in the etiology of the disease (Sonstegard and Leatherland 1976; Moccia et al. 1977). Great Lakes salmon are heavily contaminated with a variety of organochlorines, some of which have been reported to alter thyroid activity in a number of other species (Walker 1976; Jeffries and Parslow 1972; Bastomsky et al. 1976) and have been implicated in the etiology of goiter formation in Great Lakes coho salmon (Sonstegard and Leatherland 1976; Moccia et al. 1977). In this study yearling coho salmon were given diets containing Mirex and PCBs (two organochlorines found in Lake Ontario coho salmon) to determine whether these pollutants altered thyroid activity and might therefore be associated with goiter etiology in this species.

Materials and Methods

Coho salmon fingerlings (*Oncorhynchus kisutch*) were obtained from Chatsworth Hatchery Ontario, and maintained in the laboratory under a 12h light : 12 h dark photoperiod regime in continuously running and treated well water (hardness 350 mg/L; pH 8.1) at 9–10°C; they were fed daily ad lib. with a dry pelleted trout diet. The fish were maintained in the laboratory for 8 mo before commencement of the experiment.

Experimental diets were prepared using a dry starter (GRT-76-5) (Martin's Feed Mills, Elmira, Ontario). PCBs (a 1:4 mixture of Aroclor® 1242 and 1254[2]) or Mirex (a purified preparation donated by Dr T. Hallett, Canadian Wildlife Service, Ottawa) was suspended in olive oil and slowly added to the feed with continuous mixing in a commercial food mixer. Water was added to the dry mixture (30% wt/wt) and the wet diet mixture was pelleted and stored frozen at −30°C until use. An uncontaminated control diet was prepared in the same manner.

Groups of 30 fish were randomly distributed into six experimental aquaria maintained under conditions identical to those of the stock aquaria. Fish were fed daily with one of the following diets: uncontaminated control diet, 500 μg PCBs/g dry feed (high PCB), 50 μg PCBs/g dry feed (low PCB), 50 μg Mirex/g dry feed (high Mirex), 5 μg Mirex/g dry feed (low Mirex), 50 μg PCBs plus 5 μg Mirex/g dry feed (mixed diet). Fish were fed daily to about 2% of the initial body weight.

Ten fish were sampled from each aquarium 2 mo after commencement of the experiment; the remaining fish were killed after 3 mo on the diets.

The fish were each weighed and a blood sample was taken by caudal severence. The blood was allowed to clot overnight at 4°C, and the serum was separated and stored at −30°C until analysis. Analyses were made within 2 wk of collection. T4 and T3 levels in trout plasma have been shown to be stable for at least 2 mo (Milne and Leatherland, unpublished data). Serum triiodothyronine (T3) and thyroxine (T4) analyses were performed using the Ames assays as described previously (Sonstegard and Leatherland 1976). All blood samples were routinely taken in the early afternoon to avoid the possibility of circadian variability in hormone levels.

The lower jaw of each fish was removed and preserved in Bouin's fixative for histological examination of the thyroid gland. The pituitary gland was also fixed in Bouin's for histological examination.

Data on body weight, serum T3, serum T4 concentrations, and serum T4/T3 ratios were separately each subjected to a one-way analysis of variance. Predetermined individual means were compared by least significant difference.

Results

Serum Thyroid Hormones

Serum concentrations of T4 were significantly lower in fish fed the high Mirex diet after both 2 and 3 mo of the experiment ($P < 0.05$) compared with control fish. Serum T4 levels in fish fed the mixed diet were not significantly different from the control after 2 mo (Fig. 1) but were lower after 3 mo ($P < 0.01$). Fish given the PCB diets (high or low) or low Mirex diet had T4 levels that were similar to those of the controls after both 2 and 3 mo.

Serum T3 levels in control fish were significantly higher than in fish fed the high Mirex diet after both 2 and 3 mo ($P < 0.01$). After 2 mo there was no significant difference between the controls and fish fed the PCB (high and low) diets, low Mirex, and mixed diet. After 3 mo T3 levels in the mixed diet, high PCB, and low Mirex groups were significantly lower than the controls ($P < 0.01$ for high PCB and Mirex diet, $P < 0.05$ for low Mirex diet). T3 levels in low Mirex and low PCB fed fish were significantly higher than in respectively high Mirex and high PCB fed fish after both 2

[2]A 1:4 mixture of Aroclor® 1242 and 1254 closely correlates with PCB ratios found in Lake Ontario coho salmon (Nordstrom, Hallett, and Sonstegard, unpublished data).

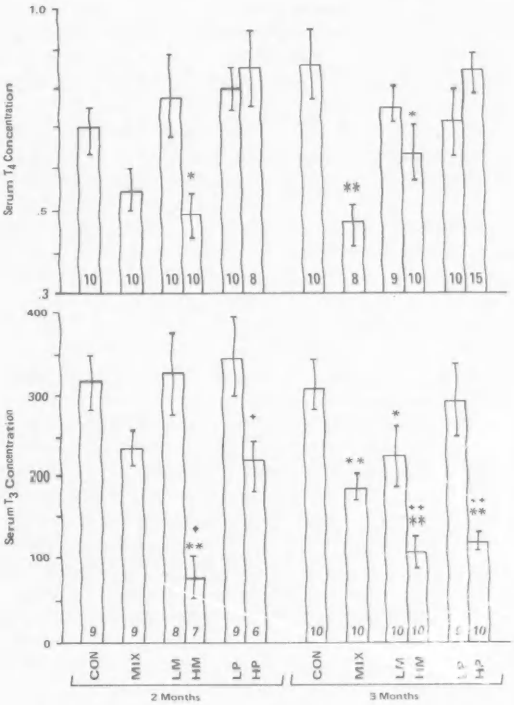

FIG. 1. Effect of Mirex- and PCB-contaminated diets on serum thyroxine (T4) and triiodothyronine (T3) levels in yearling coho salmon. Data are shown as $\mu g/100$ mL and ng/100 mL for T4 and T3, respectively (means ± SE); the number at the base of each column shows the number of samples. CON, control diet; MIX, mixed PCB- and Mirex-contaminated diet; HM and LM, high and low Mirex-contaminated diets; HP and LP, high and low PCB-contaminated diets. * and **, significantly different ($P < 0.05$ and $P < 0.01$, respectively) from the controls; + and ++, significantly different ($P < 0.05$ and $P < 0.01$, respectively) from the comparable low Mirex or low PCB diet.

and 3 mo ($P < 0.05$ after 2 mo, $P < 0.01$ after 3 mo) (Fig. 1).

The T4/T3 ratio in fish fed the high Mirex diet was significantly higher than in controls and low Mirex fed salmon after both 2 and 3 mo on the diets ($P < 0.01$). The ratio in high PCB fed fish was significantly higher than controls and low PCB fed fish after 3 mo ($P < 0.01$) (Fig. 2).

THYROID AND PITUITARY HISTOLOGY

The histological appearance of the thyroid was similar in all groups examined. The epithelium consisted of squamous or cuboidal cells surrounding homogeneous colloid which was sparsely vacuolated. There was no evidence of the overt goiter formation seen in adult Great Lakes coho salmon (Black and Simpson 1974; Drongowski et al. 1975; Sonstegard and Leatherland 1976; Moccia et al. 1977; Leatherland et al. 1978).

The cell types in the adenohypophysis were identified based on criteria described previously (Sonstegard and Leatherland 1976). There were no apparent differences in the cytology of the adenohypophysis nor of the neurohypophysis in the different groups of fish.

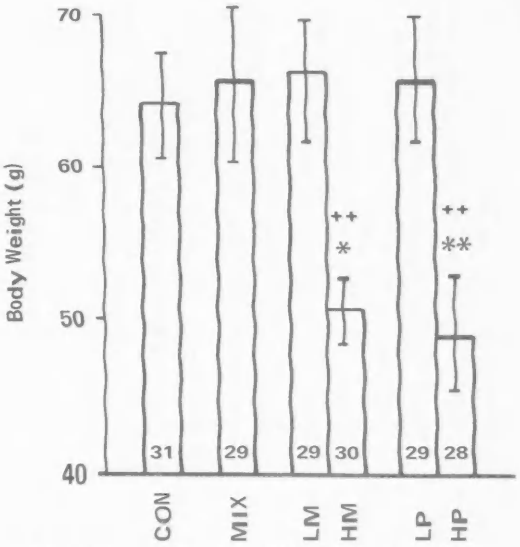

FIG. 2. Effect of Mirex- and PCB-contaminated diets on serum T4/T3 ratios in yearling coho salmon. Labeling is identical to that in Fig. 2.

BODY WEIGHT OF FISH AT THE TERMINATION OF THE EXPERIMENT

Figure 3 shows body weights of the fish at the end of the experiment. The weights of salmon fed the high Mirex (50 $\mu g/g$ dry feed) and high PCB (500 $\mu g/g$ dry feed) diets were significantly ($P < 0.05$ and $P < 0.01$, respectively) lower than the controls. Body weights of the fish fed high Mirex and high PCB diets were also significantly lower ($P < 0.01$) than fish on comparable low diets. There were no significant differences between body weights of control fish and those fed low Mirex, low PCB, or the mixed diet.

Discussion

Fish given either the high PCB- or the high Mirex-contaminated diet had significantly smaller body weights than control fish. Fish given the high Mirex diet fed poorly during the later weeks of the experiment and the low body weight in this group possibly relates to the reduced food intake. The group fed the high PCB diet, however, fed voraciously throughout the experiment and exhibited a marked aggressive feeding behavior and

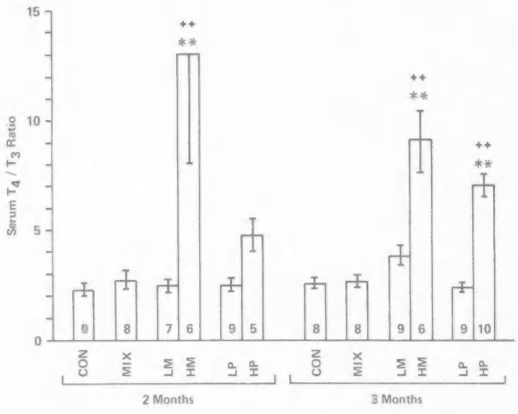

FIG. 3. Effect of Mirex- and PCB-contaminated diets on growth of yearling coho salmon. Data are shown as means ± SE; the number at the base of each column shows the total number of fish at the end of the experiment. CON, control diet; MIX, mixed PCB- and Mirex-contaminated diet; HM and LM, high and low Mirex-contaminated diets; HP and LP, high and low PCB-contaminated diets. * and **, significantly different ($P < 0.05$ and $P < 0.01$, respectively) from the controls; ++, significantly different ($P < 0.01$) from the comparable low Mirex or low PCB diet.

yet still exhibited poor growth; the low body weight may be related to inefficient conversion of food to body mass, an effect found in PCB fed mammals (Hansen et al. 1976). Low Mirex, low PCBs, and a mixture of low PCBs and low Mirex appeared to have no effect on growth.

The Mirex component of the mixed diet may have accounted in part for the significant reduction of T3 levels in fish given the mixed diet for 3 mo (Fig. 2), although the observation that T4 levels were also reduced in this group but not in fish fed diets containing either low PCBs or low Mirex suggests an interaction of these two materials on thyroid activity in this species.

Serum T4 levels were not affected by PCB-contaminated diets whereas serum T3 concentrations were lowered in salmon fed the high PCB diet for 2 and 3 mo (Fig. 2). This finding combined with the euthyroid appearance of the thyroids in these fish may suggest that the systemic conversion of T4 to T3, which is thought to be the primary mode of T3 production in mammals (Westgren et al. 1977) and rainbow trout (Milne and Leatherland 1978), is inhibited by PCBs. In mammals PCBs cause goiters (Bastomsky 1977) associated with marked decreases in serum thyroid hormone levels; the latter is thought to be caused by increasing peripheral metabolism of thyroxine, decreasing the binding of thyroxine to serum proteins, and inhibiting thyroxine secretion of the thyroid (Bastomsky 1977; Bastomsky et al. 1976; Collins et al. 1977).

The lowered thyroid hormone levels in coho fed the high Mirex or high PCB diets may be a factor involved in the low body weights in these groups or, conversely, be a response to poor growth. However, the reduced serum T3 and T4 levels in fish given the mixed diet and the reduced T3 levels in fish given the low Mirex diet (Fig. 2) without a concomitant decrease in growth in these groups suggests that the changes in thyroid secretion are not responses to reduced growth rate.

The observation that the mixed diet lowered serum T3 and T4 levels to an extent similar to that of fish fed the high Mirex diet (Fig. 2) suggests a possible synergistic effect of the low levels of Mirex and PCBs in the mixed diet. However, the T4/T3 data (Fig. 3) suggest that the effect of the mixed diet on thyroid function differed from that of diets contaminated with either high Mirex or high PCBs. The serum T4/T3 ratios in controls and mixed diet were similar, whereas in salmon fed high PCB or high Mirex diets the T3 levels were relatively more reduced than T4 resulting in a significantly increased T4/T3 ratio in these groups. The pharmacological reason for this is not known but may be related to an impairment of T4 to T3 conversion by both PCBs and Mirex (see above discussion), an effect that is not found when these materials are given individually (low diets) or combined (mixed diet) at low levels.

Although both Mirex and PCBs appeared to reduce serum thyroid hormone levels in yearling coho salmon, there was no histological evidence of thyrotroph hyperplasia, thyroid hyperplasia, or overt goiters similar to those reported in adult coho salmon in the Great Lakes (Black and Simpson 1974; Drongowski et al. 1975; Sonstegard and Leatherland 1976; Moccia et al. 1977; Leatherland et al. 1978). This may mean that PCBs and Mirex are not significant goiterogens in feral coho salmon in the Great Lakes. Conversely, these organochlorines may be maximally effective as goiterogens only in a low iodine environment similar to that found in the Great Lakes and/or at a stage in the life cycle when the thyroid is physiologically active. A further possibility is that organochlorines act synergistically as suggested by the response to mixed diets containing low levels of Mirex and PCBs, levels that as individual contaminants elicited no response. Synergism of a number of organochlorines may have a goiterogenic potency that was not demonstrable in these experiments.

Organochlorines are widespread, persistent pollutants in the biosphere. The potential of synergistic effects, as suggested by these experiments, is thus of great concern particularly since dietary controls imposed by various national food and drug administrations have been concerned with levels of individual chemicals. The consumption of a number of this diverse group of chemicals (e.g. in a meal of Lake Ontario coho salmon) each of which may be below the tolerance levels of an individual chemical may elicit a response that constitutes a distinct health hazard.

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Effect of Ozonation on Microbial Fish Pathogens, Ammonia, Nitrate, Nitrite, and BOD in Simulated Reuse Hatchery Water[1]

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COLBERG, P. J., AND A. J. LINGG. 1978. Effect of ozonation on microbial fish pathogens, ammonia, nitrate, nitrite, and BOD in simulated reuse hatchery water. J. Fish. Res. Board Can. 35: 1290–1296.

The effectiveness of ozone as a disinfectant of makeup water and its potential for treatment of recycled water in commercial reuse hatcheries was considered in this study. Comparative survival rates in water were established for four bacterial fish pathogens (*Aeromonas salmonicida*, *A. liquefaciens*, *Pseudomonas fluorescens*, *Yersinia ruckeri* [the causative agent of enteric redmouth disease]), *Bacillus polymyxa*, and a bacterium–protozoan population during batch and continuous flow ozonation. A specific microbial ozone demand was exerted during batch ozonation, and >99% mortality of the fish pathogens was observed within 60-s contact during continuous flow exposure at 1.0 and 0.1 mg O_3/L. Spores of *B. polymyxa* were resistant to a concentration of 1.0 mg O_3/L for at least 10 min. The oxidation rate for the combined bacterial–protozoan biomass closely approximated rates established in pure culture studies, with no significant difference in relative survival rates between bacteria and protozoa. Elevated carbon levels did not appear to exert a preferential ozone demand when added to suspensions of test organisms. Oxidation of carbon and nitrite by ozone was rapid at low ozone concentrations, with carbon and ammonia oxidation rates exhibiting pH-dependence. The oxidation capacity of ozone in water was greatest at elevated pH even though the measurable ozone concentration was lower.

Key words: ozone, fish pathogens, BOD, nitrite, ammonia, protozoans

COLBERG, P. J., AND A. J. LINGG. 1978. Effect of ozonation on microbial fish pathogens, ammonia, nitrate, nitrite, and BOD in simulated reuse hatchery water. J. Fish. Res. Board Can. 35: 1290–1296.

Nous avons étudié l'efficacité de l'ozone comme désinfectant de l'eau d'appoint et les possibilités qu'il offre dans le traitement de l'eau recyclée des pisciculture commerciales qui réutilisent leur eau. Nous avons déterminé les taux de survie dans l'eau de quatre bactéries pathogènes des poissons (*Aeromonas salmonicida*, *A. liquefaciens*, *Pseudomonas fluorescens*, *Yersinia ruckeri*, l'agent responsable de la maladie entérique de la bouche rouge), de *Bacillus polymyxa* et d'une population d'un mélange de bactéries et de protozoaires au cours d'ozonisations par charges successives ou à débit continu. Nous avons mis en oeuvre une demande spécifique d'ozone par les microbes durant l'ozonisation par charges successives, et avons observé une mortalité de plus de 99% des agents pathogènes des poissons en dedans de 60 s durant l'ozonisation à débit continu à des concentrations de 1.0 et 0.1 mg O_3/L. Les spores de *B. polymyxa* sont résistantes, pendant au moins 10 min, à une concentration de 1.0 mg O_3/L. Le taux d'oxydation de la biomasse bactéries–protozoaires combinés ressemble beaucoup aux taux établis au cours d'études de cultures pures, sans qu'il y ait de différence significative dans le taux de survie relatif des bactéries et des protozoaires. Des niveaux élevés de carbone ne semblent pas exercer de demande préférentielle d'ozone lorsqu'on l'introduit dans des suspensions d'organismes expérimentaux. L'oxydation du carbone et des nitrites par l'ozone est rapide à de faible concentrations d'ozone, les taux d'oxydation du carbone et de l'ammoniac dépendant du pH. La capacité oxydative de l'ozone dans l'eau est maximale à des pH élevés, même si la concentration mesurable d'ozone est plus faible.

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RECIRCULATION of rearing water in hatcheries is economically and environmentally desirable; however, it may enhance the potential for outbreaks of disease and accumulation of toxic metabolities. Since chemically treated intake and recycled water is unsuitable for fish culture, recycle hatcheries subject incoming water to sand filtration and/or ultraviolet radiation, and recycled water is passed through biological nitrification beds to reduce ammonia, nitrite, and BOD. Ultraviolet radiation is often incapable of adequately penetrating a stream of inflowing water due to turbidity (Honn and Chavin 1976), and maintenance and replacement costs are often high. Biological nitrification beds are subject to great fluctuations in efficiency depending on water quality, load, temperature, and other factors (Johnson and Sieburth 1974).

One promising alternative treatment of intake and recycled water is ozonation. Ozone has disinfecting properties, it can oxidize BOD, ammonia, and nitrite, and it rapidly breaks down in water (half-life = 15 min).

Few studies have been designed to assess ozonation in reuse hatcheries or on specific fish pathogens. Rosenlund (1975) used ozone in a hatchery system but technical difficulties precluded completion of the study. Wedemeyer and Nelson (1977) compared inactivation of two bacterial fish pathogens and viruses (Wedemeyer et al. 1978) treated with ozone and chlorine. This study was designed to assess the effectiveness of ozone to eliminate fish pathogens and reduce nitrite, ammonia, and BOD associated with reuse hatchery systems.

Materials and Methods

OZONE PRODUCTION

Ozone was produced from oxygen in the laboratory by a reconstructed, water-cooled Welsbach model C1-D corona discharge generator (Welsback Corp., Philadelphia, Pa.). A glass contact column was modified to enable syringe withdrawal of samples without interruption of the ozone flow during survival and oxidation studies. Ozone entered the sealed column of water through a sintered glass dispersion tube suspended near the base of the contact chamber. Tygon® tubing (Norton Plastics and Synthetic Div., Akron, Ohio) was threaded into the column through a "t" joint in the exit line and was sealed in place with silicon glue to prevent escape of ozone into the laboratory. A syringe was used to initiate sample flow and 1- or 2-mL samples were withdrawn by sterile 1- or 5-mL glass syringes, respectively, at 1-min intervals.

Batch ozonation studies were accomplished by ozonation of 180 mL of sterile water or buffer for 15 min at a precalibrated flow rate giving a final concentration of 1.0 mg O_3/L. Ozonation was then stopped, organisms were added, and samples withdrawn at 1-min intervals. Continuous flow studies differed in that ozone flow was not suspended after adding the organisms. Concentrations of ozone were varied by altering the flow rate of ozone through the contact chamber. The action of the ozone was stopped by immediate addition of the sample to 9 mL of 0.1% Tryptone® Broth. Ozone concentrations in water were determined by the spectrophotometric method of Schechter (1973).

PREPARATION AND ENUMERATION OF CULTURES FOR SURVIVAL STUDIES

The four bacterial fish pathogens tested, *Aeromonas liquefaciens, Pseudomonas fluorescens*, ERM, tentatively designated *Yersinia ruckeri* (Snieszko 1977), and *A. salmonicida*, were originally isolated from confirmed disease cases. The test organisms were propagated in shake flasks of Trypticase® Soy Broth (TSB) at 25°C for 18 h. Aliquots of the culture were centrifuged at 3000 × *g* for 10 min, washed once in sterile 0.02 M phosphate-buffered saline (PBS), and resuspended in sterile PBS.

Spore suspensions of *Bacillus polymyxa* were prepared following propagation on bottle slants of (TSA) for 5–7 d at 30°C. Cells were harvested, suspended in sterile PBS, heated in a water bath at 80°C for 15 min, and sonicated for 30–60 s with a Biosonik III (Bronwill Scientific, Rochester, N.Y.). The resulting suspension was centrifuged and washed 3 times in sterile PBS.

A mixed population of bacteria and biflagellated protozoans was isolated from soil and grown at room temperature on plates of 0.1% Tryptone® (1.5% Agar), with a 9-mL surface layer of 0.1% Tryptone Broth. The mixed culture was harvested after 48 h, centrifuged, washed once, and resuspended in sterile PBS.

Survival of bacterial fish pathogens and *B. polymyxa* spores during exposure to ozone was monitored by surface spread plating in triplicate on TSA. Bacteria in the mixed soil culture were plated in triplicate on 0.5% Tryptone (1.5% Agar). Serial dilutions were made in 9-mL volumes of 0.1% Tryptone Broth. Fish pathogen and soil culture plates were incubated at 25°C for 24–48 h. Protozoan survival was determined by direct count in a hemocytometer under a phase contrast microscope.

Glucose additions were made at BOD levels of 5, 10, 25, 40, 50, and 100 mg/L. Test conditions were maintained at pH 6.0 and pH 7.2 with 0.02 M phosphate buffer, and pH 8.2 and pH 9.3 with 0.0125 M borate buffer.

CARBON, AMMONIA, AND NITRITE OXIDATIONS

A 5-mL glass syringe was used to withdraw 3-mL samples at 1-min intervals, which in turn were dispensed as 1-mL replicates into precombusted 10-mL glass ampules containing 5 mL water, 0.2 g potassium persulfate ($K_2S_2O_8$), and 0.25 mL 6% phosphoric acid. The ampules were sealed and autoclaved for 4 h at 121°C to oxidize carbon to CO_2. Carbon dioxide was measured by infrared spectroscopy using the Model 0524B Total Carbon System (Oceanography International Corp., College Station, Tex.).

A 5-mL glass syringe was used to withdraw samples at 1-min intervals, which were dispensed in 2-mL aliquots for duplicate nitrate or nitrite determinations. The samples were added directly to the respective reagents to facilitate inactivation of ozone. Oxidation of ammonia as ammonium chloride (NH_4Cl) to nitrate was determined by the chromotropic acid method (APHA 1976). Oxidation of nitrite as sodium nitrite ($NaNO_2$) to nitrate was monitored as nitrite disappearance by the sulfanilic acid method (APHA 1976).

Lines in Fig. 2–10 are regression lines generated by using the means of three determinations (survival experiments) for each sample time (1 min) or the means of two determinations (nitrite, ammonia, and carbon experiments) for each sample time.

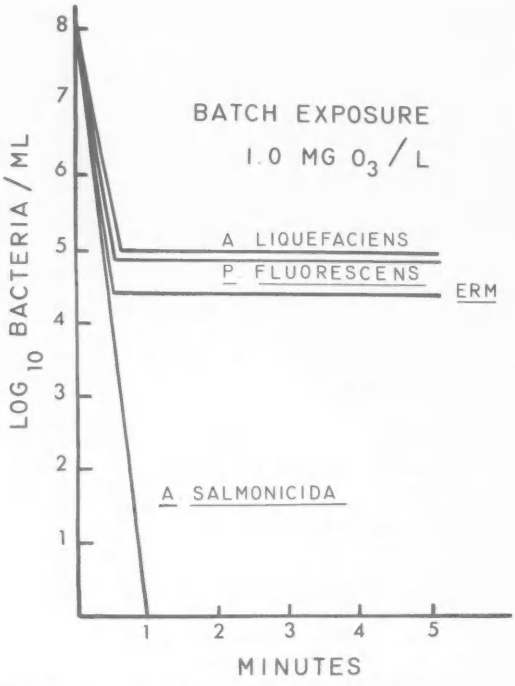


FIG. 1. Survival of *Aeromonas liquefaciens*, *Pseudomonas fluorescens*, ERM, and *A. salmonicida* during batch ozonation at 1.0 mg O_3/L in pH 7.0 distilled water.

Results and Discussion

BATCH EXPOSURE

Only *A. salmonicida* was reduced to extinction at 1.0 mg O_3/L, indicating a greater sensitivity to ozone than the other tested organisms (Fig. 1). An "all-or-none survival effect," reportedly a characteristic feature of ozone treatment (Fetner and Ingols 1956; Perlman 1969), could be used to describe the death rate curves in Fig. 1. However, the sharp breaks in the curves and lack of further kill could more likely be attributed to a specific ozone demand by organisms and by the rapid decomposition of ozone in water. "All-or-none" data have usually been gathered in some type of batch exposure system (Broadwater et al. 1973; Majumdar et al. 1973) and interpretation of batch data has supported the "all-or-none" concept. When ozone was constantly supplied in this study, even at low levels (0.05 mg/L), no "all-or-none" effect was apparent.

CONTINUOUS EXPOSURE

The rates of die-off in pH 7.0 distilled water for all the fish pathogens increased as the concentration of ozone in solution increased (Fig. 2–5). Approximately 99% mortality of dense suspensions of pure cultures of the four fish pathogens can be achieved within 60 s of contact in water at an ozone concentration of 0.15 mg/L. Death rates of the pathogens in the continuous flow studies are similar to those reported by other investigators who also failed to observe the "all-or-none" effect in inactivation studies of a variety of microorganisms (Burleson et al. 1975; McNair Scott and Lesher 1963).

Aeromonas salmonicida was the most sensitive to ozone in the continuous flow ozone studies (Fig. 6), which is contrary to data of Wedemeyer and Nelson (1977) who reported that *A. salmonicida* was more resistant to ozone than was ERM. There are no reports to explain our consistently observed sensitivity of *A. salmonicida* to ozonation, nor is there information that would explain the differences between our data and that of Wedemeyer and Nelson (1977). However, strain differences, pH differences, growth of the organism, and treatment of the suspension prior to use may affect the response to ozone.

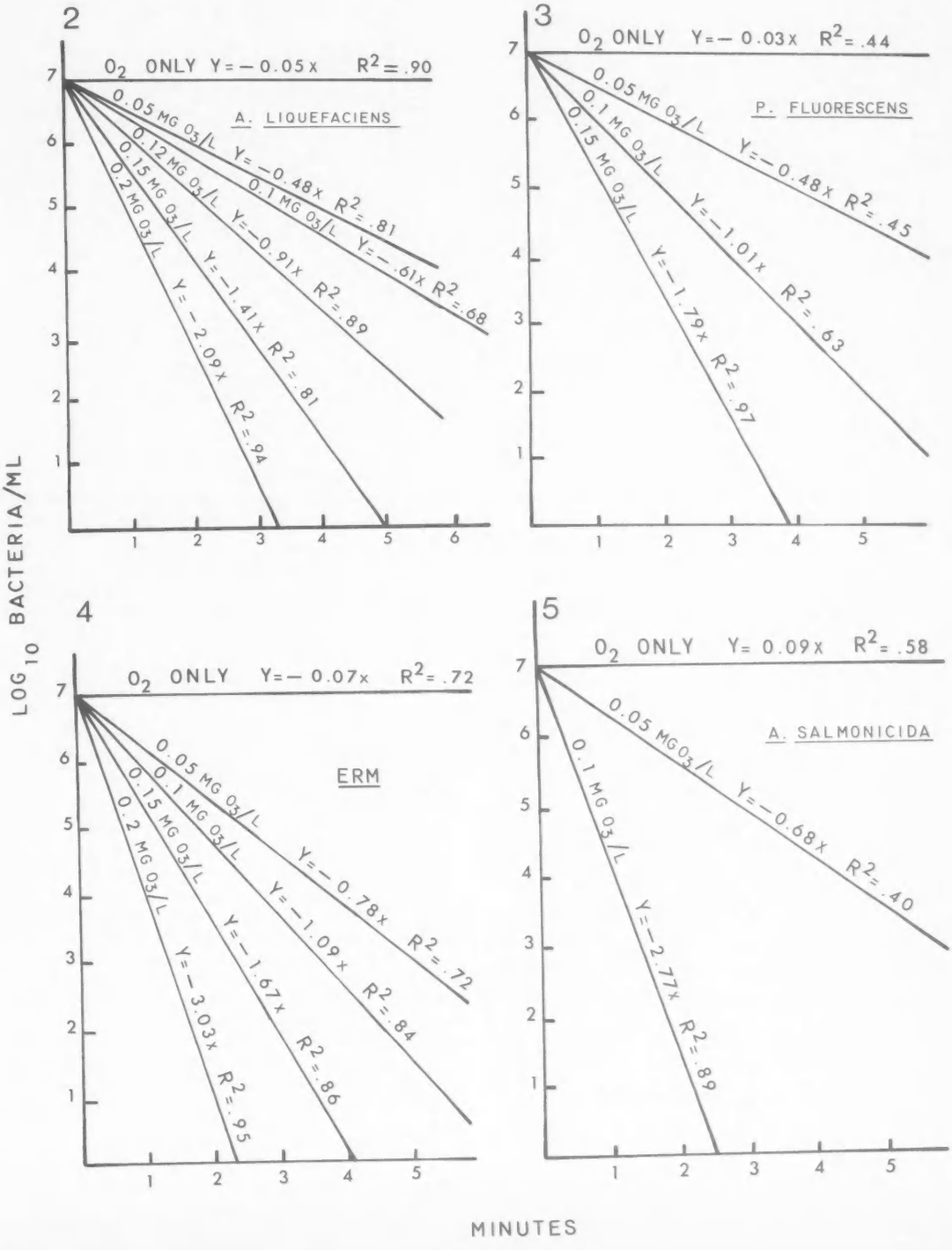
No differences in bacterial survival were detected at elevated BOD levels under the same experimental conditions of continuous flow ozonation. Studies of the effect of dissolved organic matter on survival of organisms exposed to ozone-treated water confirm that organic matter in solution may compete with microorganisms by exerting an ozone demand, resulting in less efficient disinfection of water (Broadwater et al. 1973; Dickerman et al. 1954; Ingram and Haines 1949). However, carbon levels (BOD) added were much higher than those used in this study which are nearer the BOD ranges of hatchery waters.

Spore suspensions of *B. polymyxa* were subjected to ozone as a model of the most resistant organisms. The maximum concentration of ozone that was consistently generated was approximately 1 mg O_3/L. At that level, spore viability was unaffected by continuous flow exposure to ozone for 10 min. Broadwater et al. (1973) subscribe to the "all-or-none" phenomenon with threshold concentrations established between 2.03 and 2.29 mg O_3/L for spores of *B. cereus*, *B. megaterium*, and *B. subtilus* during batch ozonations.

Since many protozoans pathogenic to fish are sporozoans (Amlacher 1970), and difficult or impossible to culture, a mixed population of bacteria and biflagellated protozoans isolated from soil was used

FIGURES 2–5.

FIG. 2. Survival of *Aeromonas liquefaciens* during continuous flow ozonation at varying ozone concentrations in pH 7.0 distilled water; FIG. 3. survival of *Pseudomonas fluorescens* during continuous flow ozonation at varying ozone concentrations in pH 7.0 distilled water; FIG. 4. survival of ERM during continuous flow ozonation at varying ozone concentrations in pH 7.0 distilled water; FIG. 5. survival of *A. salmonicida* during continuous flow ozonation at varying ozone concentrations in pH 7.0 distilled water.


2
O_2 ONLY Y = − 0.05x R^2 = .90
A. LIQUEFACIENS
0.05 MG O_3/L Y = −0.48x R^2 = .81
0.12 MG O_3/L Y = −0.91x R^2 = .89
0.1 MG O_3/L Y = −.61x R^2 = .68
0.15 MG O_3/L Y = −1.41x R^2 = .81
0.2 MG O_3/L Y = −2.09x R^2 = .94
3
O_2 ONLY Y = − 0.03x R^2 = .44
P. FLUORESCENS
0.05 MG O_3/L Y = −0.48x R^2 = .45
0.1 MG O_3/L Y = −1.01x R^2 = .63
0.15 MG O_3/L Y = −1.79x R^2 = .97
4
O_2 ONLY Y = − 0.07x R^2 = .72
ERM
0.05 MG O_3/L Y = −0.78x R^2 = .72
0.1 MG O_3/L Y = −1.09x R^2 = .84
0.15 MG O_3/L Y = −1.67x R^2 = .86
0.2 MG O_3/L Y = −3.03x R^2 = .95
5
O_2 ONLY Y = 0.09x R^2 = .58
A. SALMONICIDA
0.05 MG O_3/L Y = −0.68x R^2 = .40
0.1 MG O_3/L Y = −2.77x R^2 = .89
LOG_{10} BACTERIA/ML
MINUTES

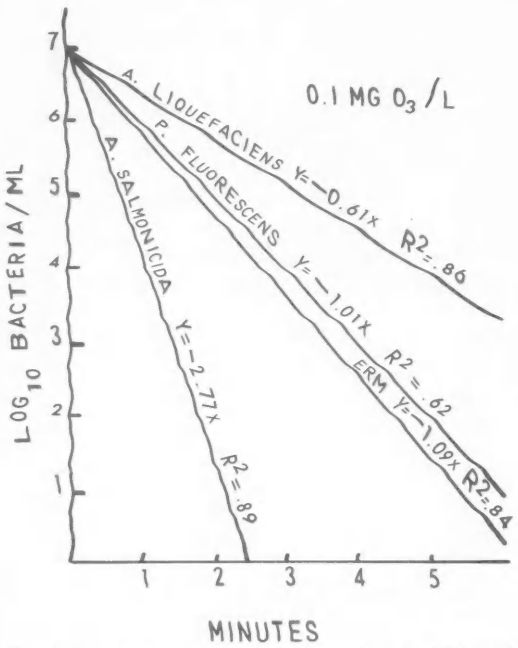


FIG. 6. Relative survival rates of *A. liquefaciens*, *P. fluorescens*, ERM, and *A. salmonicida* during continuous flow ozonation at 0.1 mg O_3/L in pH 7.0 distilled water.

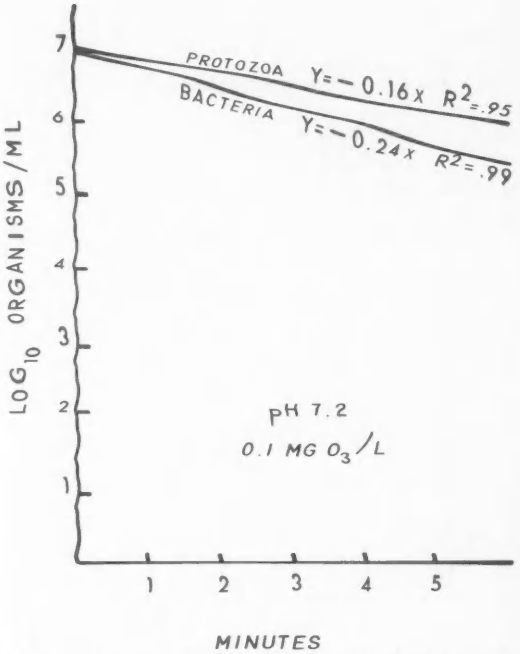


FIG. 7. Comparative survival rates of mixed bacterial–protozoan population during continuous flow ozonation at 0.1 mg O_3/L in sterile phosphate buffer at pH 7.2.

in this study to simulate ozonation effect on protozoan fish pathogens. The oxidation of the mixed culture at pH 7.2 (Fig. 7) closely approximated oxidation rates established for the pure culture studies (Fig. 2–5). Analysis of variance indicates no significant difference in the relative survival rates between bacteria and protozoa at 0.1 mg O_3/L. Reports of ozone inactivation of protozoans are limited to some early microscopic observations by Giese and Christensen (1954) and evaluations of the cysticidal potential of ozone on cysts on the waterborne agent of amoebiasis (Kessel et al. 1944; Newton and Jones 1949).

The concentrations of ozone in aqueous systems were pH-dependent (Table 1). Buffer solutions at four pH levels were ozonated for 15 min and as the pH increased, the final concentrations of ozone decreased. This variance in oxidation potential with increased pH was examined further in a series of measurements of carbon oxidation. Carbon as glucose was subjected to 1.0 mg O_3/L and oxidation rates measured in buffers at pH 6.0, 7.2, 8.2, and 9.3, and oxidation monitored by infrared carbon analysis (Fig. 8). The oxidation activity of ozone was greater at elevated pH, even though the measureable ozone concentration in solution was lower (Table 1).

Since the influence of pH seemed substantial for carbon oxidation, one of the fish pathogens, ERM, was tested in buffer solutions at the same four pH levels.

TABLE 1. Effect of pH on concentration of ozone in solution after continuous flow for 15 m.

Buffer system	pH	mg O_3/L
0.0125 M borate	9.3	0.15
0.0125 M borate	*8.2*	*0.25*
0.02 M phosphate	7.2	0.38
0.02 M phosphate	6.0	0.44

No significant differences in survival of ERM at 0.1 mg O_3/L were apparent among exposures in pH 7.0 distilled water or the different pH levels. Likewise, no significant differences in survival of the mixed bacterial–protozoan population were observed as the pH was increased to 8.2 and 9.3. Though pH had no apparent effect on rate of kill in either organism test system, carbon oxidation clearly exhibited pH-dependence. Consequently, the biocidial activity of ozone appears due to a mechanism more specific than random oxidation.

Since ammonia is toxic to fish and elimination of ammonia is essential for maintenance of fish (Honn and Chavin 1976; Spotte 1970), ammonia as ammonium chloride (NH_4Cl) was exposed to 1.0 mg O_3/L and oxidation was determined as nitrate forma-

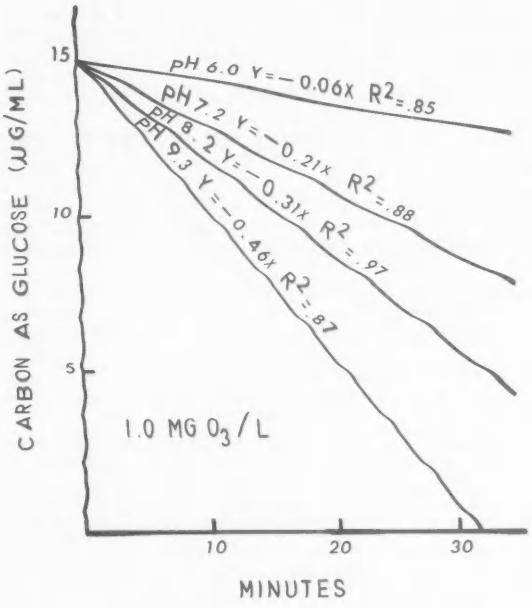


FIG. 8. Oxidation rates of carbon (as glucose) at different pHs during continuous flow ozonation at 1.0 mg O_3/L.

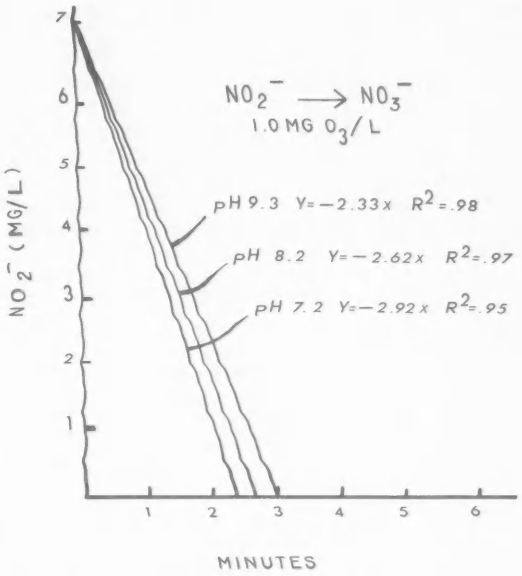


FIG. 10. Oxidation of nitrite as $NaNO_2$ during continuous flow ozonation in buffer solutions at 0.1 mg O_3/L.

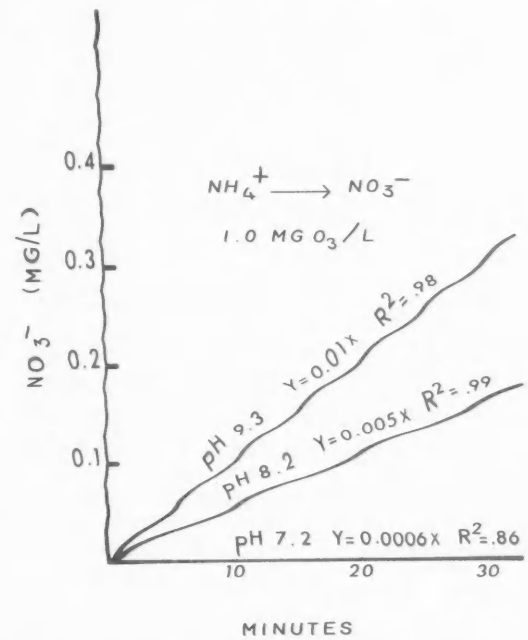


FIG. 9. Influence of pH on oxidation of ammonia as NH_4Cl during continuous flow ozonation in buffer solutions at 1.0 mg O_3/L.

tion. Oxidation of ammonia also appeared to be pH-dependent (Fig. 9). There was only negligible nitrate formation at pH 7.2. Nitrite oxidation, monitored as nitrite disappearance, was rapid at 1.0 mg O_3/L (Fig. 10), but the pH effect was statistically insignificant for the oxidation of nitrite.

Formation of free radical species due to ozone decomposition in aqueous systems (Gorbenko-Germanov and Kozlova 1973; 1974; Hoigne and Bader 1975; 1976; Peleg 1976) would account for the decreased ozone concentration in water at elevated pH (Table 1). Carbon oxidation data reveal the oxidation potential of such free radicals in water (Fig. 8).

No pH effect was evident on survival of either ERM or the mixed bacterial–protozoan population during ozonation. This suggests that the mode of action of ozone on biological matter is not necessarily influenced by free radical formation, but that oxidation readily occurs regardless of the pH of the system or the available oxidizing species.

Data for ammonia oxidation by ozone agree with work by Singer and Zilli (1975). The lack of ammonia oxidation at pH 7.2 could easily be alleviated in an aquaculture system by lime clarification.

Nitrite levels in excess of 0.2 mg/L can be related to anoxia and heavy mortalities in hatchery operations (Liao and Mayo 1972). One of the persistent problems with biological nitrification systems in reuse hatcheries has been incomplete ammonia oxidation, with subsequent nitrite accumulation. Our results show

that this problem could be efficiently resolved by ozonation.

On the basis of these results, it is easy to visualize an on-line ozone treatment system effectively alleviating the disease associated with large-scale fish culture, while efficiently oxidizing potentially toxic metabolites such as nitrite and ammonia. However, successful installation and operation of such a facility is dependent on the economics of such an investment (Majumdar and Sproul 1974), as well as provision of a mechanism for ozone removal prior to recirculation. There is concern that even low ozone "residuals," though short-lived, may cause gill adhesions and mortality in fish exposed to freshly ozonated water (Rosenlund 1975). A short-term holding tank step-ladder return flow to raceways, or passage through activated charcoal filters, are potential methods of ozone stripping. If these problems are overcome, the potential of ozone treatment of water in reuse hatcheries is both warranted and promising.

Acknowledgments

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Effect of Extended Galvanonarcosis on Behavior of Rainbow Trout, *Salmo gairdneri*, and Channel Catfish, *Ictalurus punctatus*[1]

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Yearling rainbow trout, *Salmo gairdneri*, and channel catfish, *Ictalurus punctatus*, were subjected to 0.5- and 3-h periods of narcosis using continuous direct current. Galvanonarcosis treatments decreased the immediate individual and group activity in both species of fish. Twenty-four hours after treatment, all fish except trout narcotized for 3 h recovered to levels of individual and group activity equivalent to those of untreated fish. Photonegative response was strong in both fish species and was not influenced by galvanonarcosis treatments. Trout narcotized with direct current were more vulnerable to predation than untreated fish; susceptability increased with increasing treatment periods. The potential of extended galvanonarcosis as a fish immobilization technique is strong.

Key words: galvanonarcosis, behavior, extended narcosis, direct current, rainbow trout, channel catfish, photonegative, activity, prey vulnerability, immobilization

CURRY, K. D., AND B. KYNARD. 1978. Effect of extended galvanonarcosis on behavior of rainbow trout, *Salmo gairdneri*, and channel catfish, *Ictalurus punctatus*. J. Fish. Res. Board Can. 35: 1297–1302.

Nous avons soumis des truites arc-en-ciel, *Salmo gairdneri*, et des barbues de rivière, *Ictalurus puctatus*, d'un an à des narcoses produites par un courant direct appliqué durant des périodes de 0.5 et 3 h. Chez les deux espèces, la galvanonarcose réduit l'activité immédiate individuelle et de groupe. Vingt-quatre heures après le traitement, tous les poissons, sauf les truites soumises à une narcose de 3 h, se rétablissent et ont des niveaux d'activité individuelle et de groupe équivalents à ceux des poissons non traités. Les deux espèces exhibent un phototropisme fortement négatif qui n'est pas affecté par la glavanonarcose. Les truites soumises à une narcose par courant direct sont plus vulnérables à la prédation que les poissons non traités; la sensibilité augmente en fonction de la longueur des traitements. Une galvanonarcose prolongée comme moyen d'immobiliser les poissons offre de grandes possibilités.

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INVESTIGATIONS of the effects of immobilizing fish with electricity have concentrated on short-term treatments similar to electroshocking. Past reports have shown that fish have a lower incidence of injury when immobilized with direct current instead of alternating current (Taylor et al. 1957) and continue to grow normally after electrical treatment (Maxfield et al. 1971; Ellis 1974).

The field intensities used in electroshocking immobilize fish by inducing tetanus. Vibert (1967) demonstrated that fish go through definite phases during immobilization that are directly related to the intensity of the electrical field. Galvanonarcosis, the state of muscular relaxation produced by constant direct current, occurred at a much lower field intensity than tetanus. If fish are held in a container where the length of the electric field is held constant, then the electric field could be adjusted so that the fish are immobilized in relaxed narcosis instead of tetanus.

There has been little investigation of the potential of using low intensity electric fields for extended immobilization of fish. Preliminary studies have shown that rainbow trout (*Salmo gairdneri*) can be immobilized up to 3 h with no significant effect on growth or photonegative behavior (Kynard and Lonsdale 1975). The

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purpose of this study was to investigate the effects of extended galvanonarcosis on selected aspects of the behavior of rainbow trout, *Salmo gairdneri*, and channel catfish, *Ictalurus punctatus*, to evaluate the feasibility of long-term immobilization of fish.

Materials and Methods

Yearling rainbow trout 50–87 mm standard length were obtained from the National Fish Hatchery, Alchesay-Williams Creek, Arizona. Channel catfish 45–80 mm standard length were obtained from the Arizona Game and Fish Department. Fish were held in 1600-L holding tanks for 2 wk and fed Purina Fish Chow before any experimentation.

Tap water used in all experiments was aged and aerated for at least 2 d. Conductivity was 1100–1375 mhos/cm and temperature was 12.0–18.0°C in the water used during electrical treatments.

Experimental and control fish were marked for individual recognition by cold branding (Fujihara and Nakatani 1967) and held for 72 h to check mortalities and appearance of brands. Test fish were narcotized using the procedures and apparatus described by Kynard and Lonsdale (1975). Voltage was slowly increased until 100% of the treatment group were immobilized and resting on one side. The voltage was decreased until 80% of the group remained in narcosis. Field intensities that narcotized 80% of the test fish ranged from 0.42 to 0.56 V/cm. Approximate head to tail voltages (V_{H-T}) were 2.1–4.9 V as determined by the formula $V_{H-T} = E \times L$ V where E is the electric field intensity and L is the fish length (Edwards and Higgins 1973; Kynard and Lonsdale 1975). Fish were narcotized for either 30 min or 3 h and then evaluated for effects on their behavior.

Swimming activity is a partial reflection of the general activity and motivation of a fish. The effect of galvanonarcosis on individual activity, defined as upright, horizontal swimming activity, was investigated immediately after treatment and again after 24 h. In each test, three fish were exposed to electricity and then were individually evaluated for activity after placement in a tank ($60 \times 45 \times 45$ cm; water depth 30 cm) that had a grid of 7.5-cm squares marked on the glass cover. After a 5-min acclimation period, the number of squares each fish passed through in 3 min was recorded. Control fish were treated similarly except for exposure to electricity. Student's t-test was used to compare mean activity for immediate and 24-h results between experimental and control groups.

The effect of galvanonarcosis on groups of fish was also evaluated. General group activity was defined as the number of fish in a group ($n = 5$) displaying directed upright swimming. Groups were simultaneously evaluated for activity and photonegative response in circular tanks (58 cm diam; water depth 30 cm). One half of each tank was shaded by a cover and the other half was illuminated by a 130-W overhead flood light. The number of fish from each group in the shaded half represented the photonegative response. Percent of surface light at the bottom of the tank was 42% in the lighted area and 0% in the shade.

Treatment and control groups were released in identical tanks at the shade–light interface and the initial habitat preference noted. After 3 min, both groups were observed for 15 min. A score was kept of the number of fish moving and the number of fish in the shade at the end of each minute. Then, fish were left in the experimental tanks for 1 h and observed again for 5 min. Treated and untreated groups for each test were then placed together in a tank ($60 \times 45 \times 45$ cm) and separated for evaluation after 24 h.

The null hypothesis that experimental and control groups did not differ in group activity or photonegative behavior was tested using chi-square analysis. Data from each test were compared individually and were pooled for each treatment category. Comparisons were made by chi-square analysis at the individual and pooled levels and a heterogeneity chi-square test was done for each treatment category to evaluate the trend of the individual test results (Sokal and Rohlf 1969).

Vulnerability of electrically treated trout to predation was examined by exposing equal numbers of treated and untreated fish to two largemouth bass, *Micropterus salmoides*, which were 30–35 cm total length. Predators were starved for 48 h before each test. After an electrical treatment, five test and five control fish were mixed in an 18.9-L container. The container was covered by a net and placed on the bottom of the circular predator tank (165 cm diam; water 60 cm deep). Test fish were exposed to predation 1–5 min after introduction by removing the net from the container and then removing the container from the tank. Experiments were terminated when 50% of the total group had been eaten or at the end of 0.5 h (Bams 1967; Coutant 1973). A record was kept of the number of test and control fish that remained. Results of individual tests were pooled for each treatment category since the expected frequency was less than five in each test (Sokal and Rohlf 1969). The hypothesis of no difference in vulnerability was tested on the pooled data using chi-square analysis.

TABLE 1. Individual activity of channel catfish and rainbow trout immediately after extended galvanonarcosis and after 24 h of recovery. *Significant at 0.05 probability level.

Species and group	Immediate		24-h		t	
	Test	Control	Test	Control	Immediate	24-h
Channel catfish 0.5 h	30.7±13.8 (19)[a]	96.6±18.2 (19)	69.0±9.7 (19)	62.3±15.9 (19)	2.890*	0.360[b]
Channel catfish 3 h	29.2±11.4 (16)	78.0±19.2 (16)	54.8±14.1 (15)	57.1±12.9 (15)	2.191[b]*	0.084
Rainbow trout 0.5 h	37.6±14.0 (18)	83.9±16.2 (18)	45.1±14.2 (18)	75.0±20.4 (18)	2.162	1.204
Rainbow trout 3 h	36.7±13.0 (18)	98.6±21.1 (18)	80.5±15.1 (17)	108.6±19.1 (18)	2.546[b]*	1.133

[a]Represented by the mean number of squares that individual fish passed through in 3 min ± SE. Number of fish evaluated in parentheses.

[b]t-test of means with unequal variances (Steel and Torrie 1960).

TABLE 2. Group activity of channel catfish and rainbow trout immediately after extended galvanonarcosis of 0.5 and 3 h, after 1 h of acclimation, and after 24 h. The number of fish swimming at the end of each minute was recorded and summed for each evaluation period. Five fish were evaluated in each group. The reported values represent the total of all group activity evaluations for each time period. Number of fish evaluated in parentheses. H = heterogeneity chi-square; P = probability of larger value of chi-square is less than the reported value; ns = nonsignificant chi-square value.

Observation	Test	Control	Expected		χ^2	df	P
			Channel catfish, 0.5 h				
Immediate	211 (30)	302 (30)	256.5	Total	20.848	5	.005
				Pooled	16.142	1	.005
				H	4.706	4	.50 ns
1 h[a]	57 (30)	82 (30)	69.5	Total	20.252	6	.005
				Pooled	4.496	1	.05
				H	15.755	5	.01
24 h	349 (30)	333 (30)	341.0	Total	3.891	6	.75 ns
				Pooled	0.375	1	.75 ns
				H	3.516	5	.75 ns
			Channel catfish, 3 h				
Immediate	101 (30)	388 (30)	244.5	Total	176.661	6	.005
				Pooled	168.444	1	.005
				H	8.218	5	.25 ns
1 h	37 (30)	87 (30)	62.0	Total	24.816	6	.005
				Pooled	17.887	1	.005
				H	5.930	5	.25 ns
24 h	327 (30)	351 (30)	339.0	Total	19.140	6	.005
				Pooled	0.849	1	.50 ns
				H	18.290	5	.005
			Rainbow trout, 0.5 h				
Immediate	197 (30)	360 (30)	278.5	Total	126.363	6	.005
				Pooled	47.700	1	.005
				H	78.663	5	.005
1 h[a]	94 (30)	137 (30)	115.5	Total	28.128	6	.005
				Pooled	8.004	1	.005
				H	20.123	5	.005
24 h	288 (30)	281 (30)	284.5	Total	8.290	6	.25 ns
				Pooled	0.086	1	1.00 ns
				H	8.204	5	.25 ns
			Rainbow trout, 3 h				
Immediate	193 (25)	245 (25)	219.0	Total	15.207	5	.005
				Pooled	6.174	1	.005
				H	8.034	4	.10 ns
1 h	58 (25)	116 (25)	87.0	Total	27.325	5	.005
				Pooled	19.333	1	.005
				H	7.792	4	.25 ns
24 h	175 (25)	222 (25)	198.5	Total	12.413	5	.05
				Pooled	5.564	1	.025
				H	6.848	4	.25 ns

[a]Groups observed for 5 min after 1-h acclimation. Groups observed for 15 min in immediate and 24-h evaluations.

Results

INDIVIDUAL ACTIVITY

Fish narcotized with direct current showed significantly lower levels of activity immediately after treatment (Table 1). Treated fish were more active after 24 h of recovery but the increase in activity was significant only for the 0.5-h treatment group of channel catfish. Treated fish no longer differed from controls after 24 h (Table 1), as shown by t-test comparison of activity means for experimental and control groups.

General Activity of Groups of Fish

Immediately after treatment, groups of electrically narcotized fish were not as active as untreated fish. The total and pooled chi-square values show that the difference in activity was still significant after 1 h (Table 2). Fish that had been narcotized for 0.5 h started to recover by the end of the 1-h evaluation period. The significant heterogeneity chi-square values for the data in the 1-h evaluations of this treatment group indicate that the trend of lower group activity for treated fish was no longer uniform 1 h after treatment. Heterogeneity chi-square values from the 3-h test comparisons showed that the trend of lower group activity for treated catfish and trout was still consistent 1 h after treatment.

Catfish from both treatments recovered from the effects of galvanonarcosis after 24 h. Trout narcotized for 0.5 h were fully recovered 24 h after treatment but the group activity of the 3-h treatment groups still did not equal that of control groups. The chi-square test results showed that the activity levels of these trout were not equivalent to those of the control groups. The nonsignificant heterogeneity chi-square value indicated that the trend of lower activity in treated fish was still consistent in this group after 24 h of recovery (Table 2). Catfish treated for 3 h were recovered within 24 h but the significant heterogeneity chi-square value indicated an uneven trend (Table 2).

Photonegative Behavior

Rainbow trout and channel catfish were photonegative in their immediate choice of habitat and this behavior persisted for the 24-h period (Fig. 1). Past studies demonstrated that yearling rainbow trout were photonegative (McCrimmon and Kwain 1966; Ritter and McCrimmon 1973). Galvanonarcosis treatments did not affect this behavior in either trout or catfish (Fig. 1). This supported the findings of Kynard and Lonsdale (1975).

Vulnerability to Predation

Electrically narcotized fish were more vulnerable to predation than controls and vulnerability increased with the duration of treatment (Table 3). More trout from the 0.5-h exposure group were eaten than from the untreated group but the chi-square comparison of the pooled data from 10 individual tests was not significant. In five of six individual tests, more trout narcotized for 3 h were eaten than control fish (Table 3). The pooled chi-square values clearly indicate the increased vulnerability of this group (Table 3). Data from predation tests on catfish were inconclusive because of a procedural bias that was corrected in tests on trout. In the channel catfish predation experiments, treated and control fish were introduced to the predator tank at the surface. Bass predators would consume catfish before they had time to orient within the predator tank. The procedure was modified for tests on trout so that all prey were released at the bottom of the predator tank. This permitted trout time to orient within the tank before being subjected to predation.

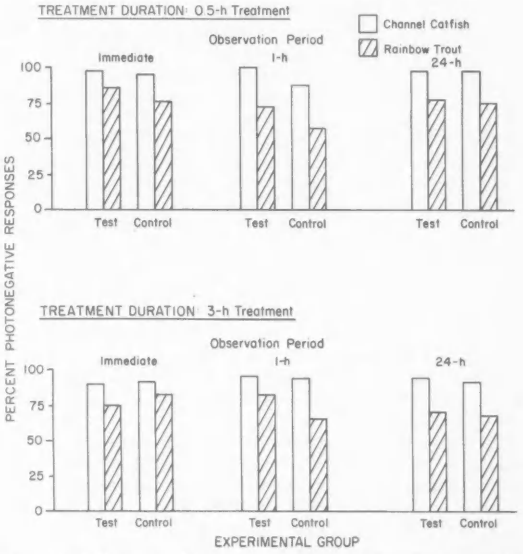

FIG. 1. Photonegative response of treated and control fish at three evaluation periods after galvanonarcosis for 0.5 h and 3 h. Sample sizes the same as in group activity. Percentages based on a total if all fish in each group had been in the shade at the end of each minute for the total duration of the observation period.

TABLE 3. Numbers of treated and untreated rainbow trout remaining after exposure to predation for 0.5 h or until one half of the total group remained.

	Treatment duration			
	0.5 h		3 h	
	Observed	Expected	Observed	Expected
Test	24	30.5	9	16.0
Control	37	30.5	23	16.0
χ^2		2.77		6.13
P		<0.10		<.025

Discussion

The group activity index did not reflect the observed differences in behavior of treated and untreated trout in the immediate evaluations of the 3-h galvanonarcosis treatments. Four individual treatment chi-square comparisons showed no difference in the group activity of treated and control fish although the groups differed behaviorally (Table 4). This makes the rejection of the null hypothesis based on the pooled and total values

TABLE 4. Individual test results of group activity for rainbow trout immediately after 3 h of galvanonarcosis. H = heterogeneity chi-square; P = probability of larger value of chi-square; ns = nonsignificant chi-square value.

Test	Control	Expected		χ^2	df	P
43	46	44.5		0.101	1	.90 ns
32	70	51.0		14.157	1	.005
32	40	36.0		0.889	1	.50 ns
39	41	40.0		1.050	1	.90 ns
47	48	47.5		0.011	1	.95 ns
193	245	219.0	Total	15.208	5	.005
			Pooled	6.174	1	.005
			H	8.034	4	.10 ns

questionable. During immediate evaluations, treated trout displayed bursts of upright swimming and opened their gills as if coughing. Swimming action of control fish was more continuous and without opercular flaring. The measured group activity index of treated fish did not change significantly after 24 h, yet their observed activity was not equivalent to that of control groups. It appeared that trout treated for 3 h still suffered side effects even after recovery for 24 h.

An additional measure of group activity would have been helpful in the analysis. Control fish were observed to cluster more during the immediate evaluations than treated fish. Test fish would not start responding as a group until the end of the evaluation period. This clustering behavior was evident in both experimental and control groups after 24 h. Quantifying this behavior may be a better way to evaluate the effect of electrical treatment in this case.

It appeared that extended galvanonarcosis affected the muscles more than the sensory system. Activity evaluations showed that the motor activities were definitely depressed in fish immediately after treatment. Treated fish were able to detect habitat differences as indicated by the study of photonegative response. In the predation tests, treated fish did not react to predator approach or disperse to the surface as quickly as control fish. The depression of motor activity in electrically narcotized fish may be due to an extreme lactate increase and an oxygen debt incurred while under galvanonarcosis. Schreck et al. (1976) found that lactate increased rapidly in rainbow trout that had been electroshocked. They observed that trout responded to electroshock as if they were suffering from extreme muscular exertion and/or hypoxia. It is not known whether fish experience these same physiological changes under galvanonarcosis.

Mortalities and delays in recovery time in fish subjected to galvanonarcosis have probably been a result of exposure to voltage gradients greater than the range needed to produce immobilization. Adams et al. (1972) used voltage gradients of 1.5–3.6 $V \cdot cm^{-1}$ to narcotize 65–85 mm *Notropis cornutus* for 10–25 s. Fish held in galvanonarcosis did not always recover to an upright position within 120 s, and those that took longer often died. Although Adams et al. (1972) found a correlation between recovery time and energy density, the voltage gradients were well in excess of those needed to narcotize similar sized fish in our study and in Taylor et al. (1957). Injuries from direct current are probably a direct result of tetanic levels of electricity. This seemed to be the case in Spencer's (1967) and Adams' et al. (1972) studies, since in both studies the voltage gradients were close to 3.5 $V \cdot cm^{-1}$. Data from our study, Taylor et al. (1957), Vibert (1963), Ellis (1974), and Kynard and Lonsdale (1957) indicate that direct current gradients below 1.5 $V \cdot cm^{-1}$ cause no injury to electrically treated fish.

The duration of the effects of extended galvanonarcosis on the behavior and physiology of treated fish might be reduced by modifying the electric field applied. Halsband (1955, 1967) reported that treated fish recovered to normal values of intensity of metabolism within 70 min after galvanonarcosis while average recovery time for fish treated with condensor charges was 20 min. The initial exciting effect an electric current has on the intensity of fish metabolism changes into a paralyzing effect when the period of electrical treatment is prolonged as well as when the current density is increased (Halsband, 1955). We have found that fish narcotized up to 3 h by direct current show no sign of behavioral side effects after 24 h. Decreasing the field intensity after fish have reached the galvanonarcosis stage and use of condensor charges might decrease the effects and recovery time of fish immobilized by extended galvanonarcosis and might permit treatments longer than 3 h.

Chemicals are often used to immobilize fish for handling but the concentrations needed to produce narcosis for long periods can be close to lethal levels (Nelson 1953; McFarland 1959; Bell 1964). Fish have been anesthetized up to 12 h with methylparafynol without demonstrable long-term effects (McFarland 1959). However, it is not easy to monitor concentrations during long periods of immobilization, and the state of chemically induced narcosis can not be terminated easily if there are complications. Also, chemical anesthetics affect the physiology and behavior of fish (McFarland 1959; Wedemeyer 1970; Crowley and Berinati 1972) with some effects lasting up to 5 d after recovery (Goddard et al. 1974).

Our study suggests that galvanonarcosis has strong potential as an alternative to chemical anesthetics for long periods of fish immobilization. Immobilization techniques are usually employed in handling operations such as marking and transport to minimize the chance of injury (Hartley 1967). Galvanonarcosis can be used to safely immobilize fish up to 3 h. There are definite immediate effects on fish behavior that were not evident in the study of Kynard and Lonsdale (1975) but these were not apparent after 24 h. Electricity can be adjusted and monitored easily so voltages needed to pro-

duce narcosis can be regulated by an inexperienced person.

Acknowledgments

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Model for Examining Early Ocean Limitation of Pacific Salmon Production[1]

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A computer simulation model was used to examine growth and survival of all major British Columbia salmon stocks during their first 6 mo of ocean life. Factors included in the calculations were the space–time distribution of zooplankton production, timing of salmon ocean arrival and migration especially as regards overlaps between stocks, feeding and growth in relation to food availability, and mortality rates in relation to body size. It is concluded that ocean limitation of production is unlikely unless only a small fraction of total zooplankton production is available to the salmon. The model emphasizes several critical uncertainties about the marine biology of salmon: rates of replenishment of near surface zooplankton stocks (where most salmon feeding occurs) from deeper water have not been adequately measured, and the functional response of salmon to prey density is not understood. There is inadequate data on the relationship between body size and mortality rate, and migration patterns of juvenile fish have not been documented precisely enough. Hopefully some of these uncertainties will be resolved through the salmon enhancement programs.

Key words: salmon, population limitation, juvenile marine production, simulation model

WALTERS, C. J., R. HILBORN, R. M. PETERMAN, AND M. J. STALEY. 1978. Model for examining early ocean limitation of Pacific salmon production. J. Fish. Res. Board Can. 35: 1303–1315.

A l'aide d'un modèle de simulation par ordinateur, nous analysons la croissance et la survie de tous les principaux stocks de saumons de la Colombie-Britannique durant leurs premiers 6 mois en mer. Les facteurs introduits dans les calculs sont la distribution spatiale et temporelle de la production de zooplancton, la chronologie de l'arrivée des saumons dans l'océan et leur migration, surtout en ce qui concerne le chevauchement des stocks, l'alimentation et la croissance par rapport à la disponibilité de la nourriture, et les taux de mortalité en fonction de la grosseur du corps. Nous concluons que la limitation dans l'océan de la production est peu probable, à moins que les saumons n'aient à leur disposition qu'une petite fraction de la production zooplanctonique totale. Le modèle fait ressortir plusieurs incertitudes critiques concernant la biologie des saumons en mer : les taux de renouvellement des stocks de zooplancton du voisinage de la surface (où a lieu la plus grande partie de l'alimentation des saumons) à partir des couches profondes n'ont pas été adéquatement mesurés, et on ne connaît pas la réponse fonctionnelle des saumons en relation avec la densité des proies. Les données sur la relation entre la grosseur du corps et le taux de mortalité sont inadéquates et les migrations des jeunes n'ont pas été démontrées avec suffisamment de précision. Il est à espérer que les programmes de mise en valeur du saumon apportent une réponse à quelques-unes de ces questions.

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ENHANCEMENT programs for anadromous salmonids have concentrated primarily on restoring and improving freshwater production of juvenile salmon. It has been a tacit assumption that the ocean has adequate capacity to produce more fish. This assumption is supported by two kinds of evidence: (1) salmon abundances were historically much higher, and the ocean probably has returned at least twice as many salmon per year as it presently does; and (2) food chain efficiency calculations based on plankton production data for the North Pacific Ocean suggest that salmon use only a small fraction of the food production available to them (LeBrasseur 1972; Sanger 1972; Rothschild 1972).

There are two grounds for questioning the assumption of adequate ocean capacity: (1) statistical — a few stocks show significant decreases in smolt–adult survival as smolt output increases (Peterman 1975, 1978), suggesting an upper limit on the number of returning adults no matter how many juveniles head downstream; and (2) functional — juvenile salmon entering the ocean are restricted to (or at least must pass through) a narrow coastal zone, so limitation could occur in this zone even if the ocean as a whole can support unlimited numbers of larger, more mobile older fish.

Unfortunately, estuaries and coastal marine areas are exceedingly difficult places to do salmon research, so only a few field studies have examined potential limiting factors for juvenile salmon in these environments (Bailey et al. 1977; Kaczynski et al. 1973; Manzer 1969; Mason 1974; Kask and Parker 1972; Parker 1971; Reimers 1973; Dunford 1975). These studies provide important data on the ingredients of the problem, but do not provide a clear overview of the interplay of factors such as timing of ocean arrival and migration of fish, food chain relationships, and the mixing of very large numbers of small fish of many stocks that can occur in areas like the Strait of Georgia.

Some sort of synthetic model is essential to provide an overview of the coastal production system, in terms of potential limits for salmon, and the purpose of this paper is to present a first attempt at this synthesis. In collaboration with a group of scientists (Table 1), we have attempted to develope a simulation model to look at roughly the first 3 mo of ocean life of all major blocks[2] of salmon entering Pacific waters from British Columbia rivers. Our objective is not to make firm predictions of ocean capacity; existing data are not sufficient to do that confidently. Rather, we hope to (1) demonstrate that large-scale, relatively detailed predictions are feasible, and (2) define more precisely the information gaps that must be filled to eventually make those predictions believable. Ultimately, any predictions about ocean rearing capacity will be tested by the enhancement program through actual attempts to increase abundance; scientifically we should welcome the oppor-

[2]By a block we shall mean all the fish of one species entering the ocean from one river; normally the block will consist of fish from several racial stocks.

TABLE 1. Scientists contributing data and ideas to the model described in this paper.

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J. R. Brett	Department of Fisheries and the Environment, Fisheries and Marine Service, Pacific Biological Station, Nanaimo, B.C.
D. M. Eggers	Fisheries Research Institute, University of Washington, Seattle, Wash.
F. J. Fraser	Department of Fisheries and the Environment, Fisheries and Marine Service, Vancouver Laboratory, Vancouver, B.C.
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TABLE 2. Major blocks of juvenile salmon included in the coastal model.

Block name	Mean time of arrival at ocean	SD of arrival (d)	Duration of stay in first marine area (d)	Number entering ocean (10^6)	Mean weight at time of arrival (g)
Fraser sockeye	May 5	6	0	50	6
Skeena sockeye	June 1	6	0	40	5
Fraser pink	Apr. 20	2	120	200	.32
Central coast pink	May 15	4	30	200	.32
Fraser chum	Apr. 20	6	60	30	.35
Central coast chum	May 15	4	30	10	.35
Fraser coho	May 1	4	60	2	10
Skeena coho	June 15	4	30	4	10
Fraser chinook	Apr. 15	4	60	20	1–4
Skeena chinook	May 10	8	30	10	1–4

tunity to cast existing data and beliefs into predictions so that we can learn from the mistakes that will inevitably result.

Components of Production

The model that we describe in this section does not pretend to capture all of the biological and physical interactions that might result in limits to salmon abundance. Instead, we have attempted to incorporate only the most basic ingredients of early ocean production:

1) the space–time distribution of zooplankton production and availability along the coast;

2) rations and growth of young salmon in relation to body size and food availability;

3) salmon mortality rates in relation to body size (presumably largely due to size-selective predators);

4) timing of ocean arrival and migration along the coast.

Thus we have not explicitly represented primary productivity and nutrient dynamics, life histories and species composition of food organisms, and specific mortality agents such as disease, parasites, birds, and other fishes.

To perform computer bookkeeping, it is necessary to divide the system into discrete units. For temporal effects, we divided the period from March 15 to September 15 into 3-day steps, and the computer bookkeeping updates all state measures (food supplies, fish numbers, body sizes, spatial locations) every step. Spatial variation is represented by dividing the coast from the mouth of the Fraser River northward into 50 strips, each 20 km in north–south length and 37.5 km wide. Each strip is assumed to have its own distinct and nondispersing zooplankton population, and patchiness within the strip is not explicitly represented. Ten major blocks of juvenile salmon are represented (Table 2), and each block is divided into 10, 3-day subunits based on time of arrival at the ocean.

The following subsections describe the four basic model ingredients listed above, with emphasis on quantitative assumptions, data sources, and key uncertainties. Model predictions are discussed in context with particular assumptions, rather than in a separate "results" section, to show more clearly how each part of the model affects its overall behavior.

Zooplankton Production and Availability

A complex array of food organisms is available to and used by salmon in the coastal environment, ranging from various life stages and species of calanoid copepods through euphausids to fish larvae and even ctenophores. It would be practically impossible to represent the dynamic response to salmon predation of each species in this array, even if food selection responses of the fish were well understood; far too many life history parameters would have to be estimated, yet even one serious estimation error (e.g. euphausid body growth rates too high) could seriously distort the whole food supply prediction. Therefore we decided to use only the simplest measure of food supply, total zooplankton biomass in each spatial strip along the coast, and to roughly portion this biomass into 90% small ("copepod") and 10% large ("euphausid") particle size categories. We initially divided biomass into four size categories ("copepods," "small euphausids," "large euphausids," "fish larvae"), but we were not able to establish differential production and utilization rates for even these categories.

We began the food analysis with three serious misconceptions about zooplankton production along the British Columbia coast: (1) the seasonal cycle of total zooplankton abundance is closely related to primary productivity, in some sort of predator–prey cycle; (2) seasonal zooplankton peaks first appear in southern latitudes, then move northward along the coast; and (3) zooplankton is much more abundant nearshore than offshore.

Figure 1 shows total zooplankton abundance in 20–400 m and in the top 20 m at one station in the Gulf of Georgia, visually averaged for 1965–68. Surface standing crop does indeed peak near the time of maximum primary production as reported by Stephens et al. (1969), but this corresponds to the time of lowest overall zooplankton standing crop. Apparently several

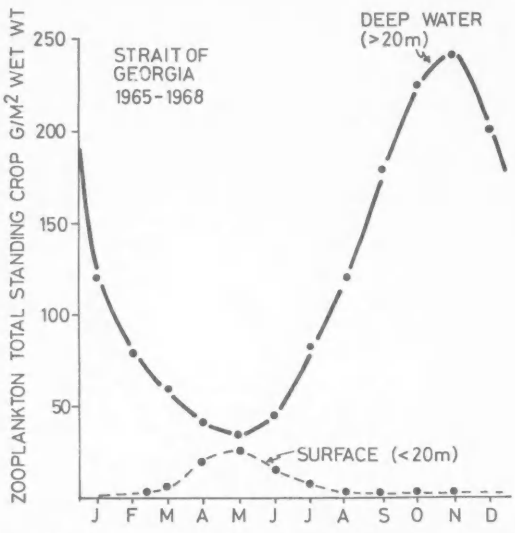

FIG. 1. Average zooplankton standing crops at Station 1, Gulf of Georgia, 1966–68. (from Stephens et al. 1969, figures 5a and b).

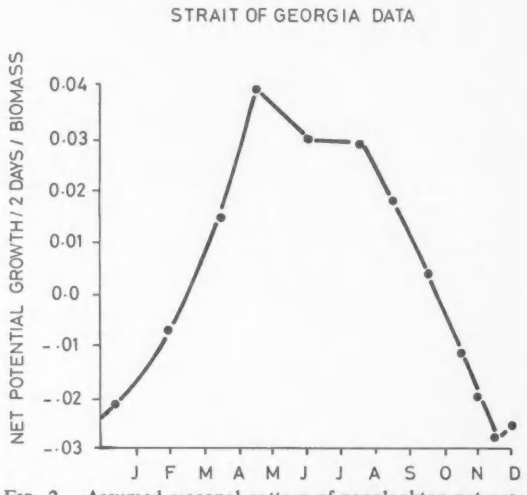

FIG. 2. Assumed seasonal pattern of zooplankton net production in British Columbia coastal areas. See Table 3.

species, like *Calanus plumchrus*, migrate into surface waters in spring, thereby taking advantage of high primary production, but then move back down into very deep water in summer (Fulton 1973). Yet young salmon are believed to feed mostly in surface waters (Straty 1974). These observations suggest that vertical migration and mixing rates may be much more important determinants of potential salmon production than are overall biological production rates of the food supply.

To make predictions about increased salmon abundance, we must look beyond present seasonal patterns of zooplankton availability; more salmon may lead to higher zooplankton mortalities, and the resulting changes in standing crop (and food available) can be predicted only by taking zooplankton growth and mortality rates into account. Assuming that specific zooplankton growth rate per surface area is roughly proportional to primary production as reported in Stephens et al. (1969), and assuming current salmon abundance and rations (next subsection), we can crudely reconstruct seasonal changes in net zooplankton production (Table 3, Fig. 2). Apparently even in the Strait of Georgia, current salmon numbers can account for only a small fraction of total zooplankton mortality, though there may be a significant impact on the surface (0–20 m) standing crop. The key uncertainty is how fast the surface zooplankton biomass pool will be replen-

TABLE 3. Standing crop and production component assumptions used to estimate seasonal zooplankton dynamics.

	Total biomass g/m²	Proportion of population in top 20 m	Assumed P/B per day based on primary prodn.	Assumed prodn. $(P/B) \times B$ per day	Assumed prodn. $(P/B) \times B$ per mo	Total mortality	Assumed present salmon consumption per month	Non salmon mortality per month	Net prodn. per m² corrected for present salmon removals per mo	Net prodn. per m² corrected for present salmon removals per biomass mo
Jan.	120	0	0	0	0	40	0.3	39.7	−40	−0.33
Feb.	80	0.005	0.008	0.64	19.2	34	1.5	32.5	−13	−0.16
Mar.	65	0.077	0.02	1.3	39.0	44	3.0	41.0	−2	0.03
Apr.	60	0.33	0.05	3.0	90.0	90	15.0	75.0	15	0.25
May	60	0.43	0.07	4.2	126.0	118	30.0	88.0	38	0.63
June	68	0.21	0.03	2.0	60.0	40	15.0	25.0	35	0.51
July	88	0.08	0.025	2.2	66.0	31	9.0	22.0	42	0.48
Aug.	123	0.024	0.025	3.0	90.0	33	6.0	27.0	63	0.51
Sept.	180	0.008	0.02	3.6	108.0	63	3.0	60.0	48	0.27
Oct.	225	0.004	0.018	4.05	121.0	106	1.5	104.5	16	0.07
Nov.	240	0.002	0.015	3.6	108.0	148	0.6	147.0	−40	−0.16
Dec.	200	0	0.005	1.0	30.0	110	0.3	110.0	−80	−0.4

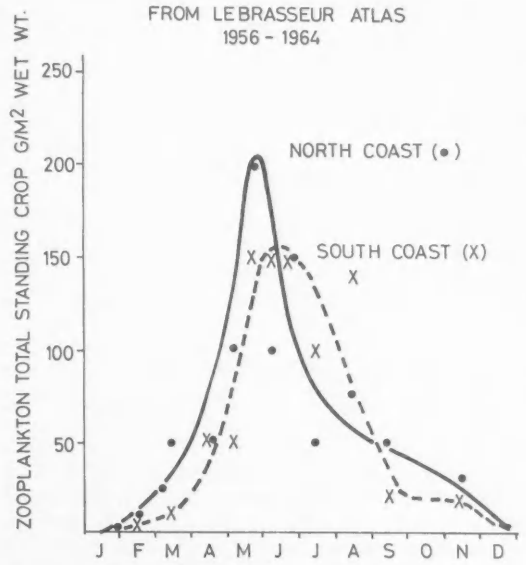

FIG. 3. Seasonal zooplankton abundance within 200 km of the British Columbia coast. "South" coast is from the north top of Vancouver Island southward.

ished from below when it is being heavily predated; for the model, we assumed that observed surface/deep water ratios (Fig. 1) will be reestablished daily. More precisely, we assume that removals by salmon during any day will be replaced from deep water that night, so as to maintain the surface proportion implied by Fig. 1. It must be emphasized that there are no data to support this assumption. Thus the model supposes that there may be severe competition among juvenile salmon for the surface standing crop present at the start of any day, provided that salmon abundance is increased sufficiently, though the total zooplankton standing crop (0–400 m) may remain virtually untouched.

Spatial variation in zooplankton stocks is certainly great, but this variation is not clearly correlated with latitude or distance offshore. LeBrasseur (1965) has summarized North Pacific zooplankton data for 1956–64, and a rough averaging of his data for the northern British Columbia coast (Queen Charlotte Islands to Alaska) vs. the south coast (mostly west of Vancouver Island) does not show any clear differences in seasonal trends (Fig. 3). Unfortunately these samples were collected with 150-m vertical tows. If more northern vertical distributions are similar to the Strait of Georgia, then 150-m tows have underestimated peak surface concentrations and have failed to capture deepwater maxima in the fall. Thus we decided to ignore the possibility of lower northern (and offshore) zooplankton populations (compared to the Strait of Georgia), and we assumed for the model that the entire coast has the standing crop-production pattern estimated in Fig. 1 and 2. Smaller scale spatial variation (river mouth effects, local upwelling near islands, etc.) is represented only in the salmon feeding rate calculations (see below).

In summary, a fish moving northward through our 50 simulated coastal strips would see food supply variations due to three factors: (1) seasonal changes in each strip of depth distributions of food organisms; (2) simultaneous presence in each strip of other fish competing for near surface food; (3) cumulative effects on food biomass in each strip caused by fish that passed through it earlier. We expected to see pronounced cumulative effects across several adjacent strips as each fish block moved northward; early running fish (near the front of the block) might see more food than latecomers that would be several strips behind. On the other hand, we expected that timing differences between blocks would result in enough spatial separation to allow food supplies to recover between waves of moving fish, so interblock competition would be minimal. As the following sections will indicate, these expectations were only partially realized, and only when we massively increased simulated salmon abundance.

Salmon Feeding and Growth

Given estimates of potential food supplies over time, the next problem is to predict how much will be eaten and converted into body growth. At least two distinct approaches to this problem are possible. First, we could do an experimental components analysis (Holling 1966) where we model the feeding of the fish based on volume searched, reactive distances, and probability of capture. This approach has been used to model salmon feeding in planktonic environments (Eggers 1975, 1977), and results in predictions of amounts consumed which could then be converted to maintenance and growth. We rejected this approach because it was felt that salmon are probably not searching at random, but more likely search for patches of high concentration and then feed intensively in those patches; schooling may also be important (Eggers 1976). We chose a second, more directly empirical approach based upon laboratory studies of meal size, maintenance requirements, and feeding rate as a function of food availability (LeBrasseur 1969; Brett 1976).

The feeding and growth model uses the concentration of available food of two size-classes (copepods, euphausids) in grams per cubic metre for the upper 20 m of water. We assume that fish weighing >8 g can feed on both sizes of food, fish <8 g can feed only on the smaller food class. The proportion of the maximum ration actually consumed is calculated as a function of food availability. The best available direct data on feeding of juvenile salmonids in relation to food availability come from Parsons and LeBrasseur (1970); Fig. 4 is a reproduction of their data. The problem with these data, as several workers have noted, is that food densities 10 times higher than that found in the British Columbia coastal waters were required to produce

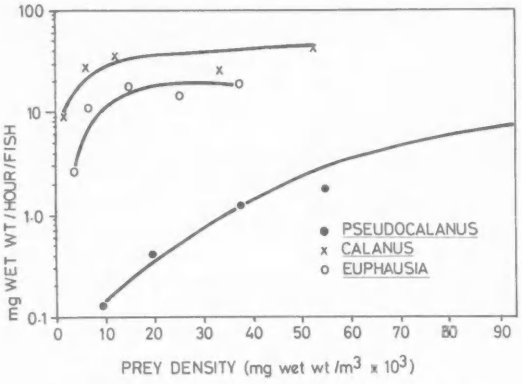

FIG. 4. Food consumption plotted in relation to food availability (from Parsons and LeBrasseur 1970).

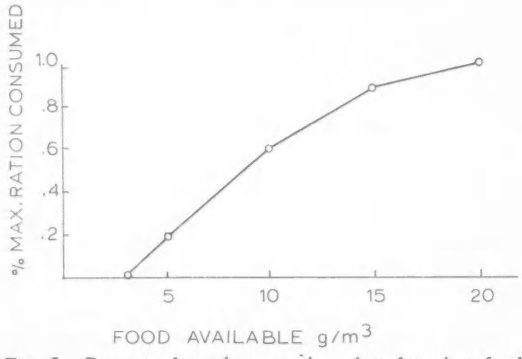

FIG. 5. Percent of maximum ration plotted against food availability. Food availability is calculated as 10 times the mean concentration.

maximum feeding rates. Our conclusion from these data and the data on food availability (discussed in the previous section) is that the salmon must be feeding in patches of substantially higher food concentration than represented by average plankton tows. We have assumed that the food concentration that the salmon see is 10 times higher than the average. We used the Parsons and LeBrasseur data to get the form of the functional response, and substituted on the Y axis the percentage of the maximum ration, producing a relationship shown in Fig. 5. The maximum ration is then calculated as a function of body weight, using the exponential function shown in Fig. 6. This function was chosen to agree with measurements by Brett (1974, 1976).

Multiplying maximum ration times proportion of maximum ration obtained yields the total intake rate, in percent of body weight, per fish. The total impact of all salmon in a given spatial area is then summed to produce the total grams of food per cubic metre potentially consumed. Because this total was potentially greater than the available food, we modeled the daily dynamics of fish feeding as a depletion attack process.

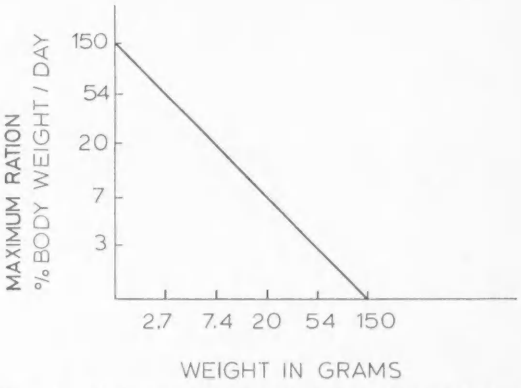

FIG. 6. Maximum ration (percent body weight per day) in relation to body weight. Data source described in text.

The negative bionomial distribution was chosen to describe this process (Griffiths and Holling 1969). If E is the amount of food potentially consumed per day, and A is the amount available, the proportion of the available food not eaten (NE) is calculated as

$$NE = \left[1 + \left(\frac{E}{A \cdot k}\right)\right]^{-k}$$

where k is a constant that Holling (personal communication, University of British Columbia) argues should usually be near 1.0. The amount actually eaten then becomes $A - NE \cdot A$. When E is much less than A, then the amount actually consumed is very close to that calculated from the functional response in Fig. 5.

The above calculations produce the amount of food consumed in percent of body weight per individual fish, and the amount of food consumed per cubic metre. When the fish are big enough to eat both sizes of food, it is assumed that the consumption of each food size-class is proportional to its abundance. Biologically we would expect that larger fish would eat a higher proportion of the large food items, but we made the simpler assumption because contradictory evidence was not available.

Fish are assumed to apply 30% of food consumed above maintenance level (Fig. 7) to growth. There is a substantial literature (Paloheimo and Dickie 1966a, 1966b; Kerr 1971) that formulates fish growth in considerably more detail, but these models require much more data. We believed that more detail in the formulation of the fish feeding model would not result in more accurate predictions.

RESULTS OF FEEDING AND GROWTH MODEL

During most of the simulated year, food is abundant enough that fish attain near maximum ration. The growth of the five species is shown in Fig. 8. (Growth is shown only in the 1000-km coastal zone represented in the model.) Under maximum ration, the model pre-

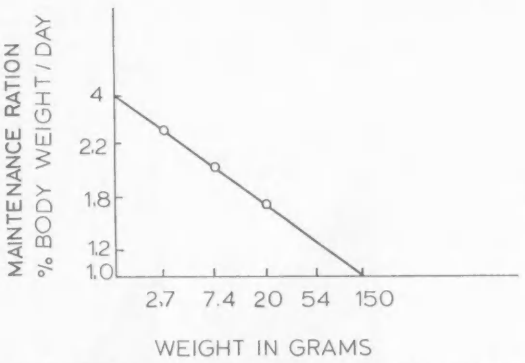

FIG. 7. Maintenance ration (percent body weight per day) in relation to body weight. Data source described in text.

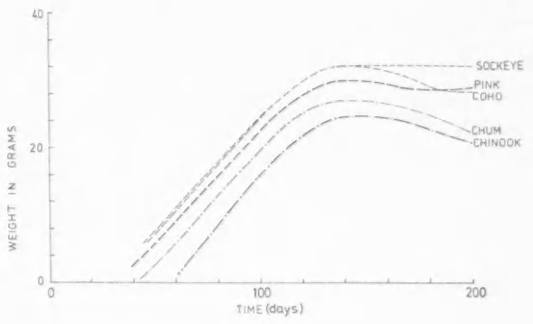

FIG. 8. Simulated body weight of the five salmon species plotted against the time of year, in days from March 15th.

dicts linear growth. This is because there is a general tendency towards exponential growth in weight, exactly countered by a negative exponential change in maximum ration measured as a percent of body weight. The maintenance ration is also negatively exponential with respect to body weight. This is not totally realistic, but for the purposes of this model we believe it is sufficient.

Data on early growth rates of juvenile salmon in the marine environment are few. Size distributions over time have been measured frequently, but are difficult to interpret since they represent a mixture of ocean residence times; also, small fish are difficult to tag and are seldom recovered. Early maturing species (chum and pink) should probably grow faster than sockeye, chinook, or coho. In the absence of better data, we have made all feeding and growth size dependent, but not species dependent. The declining body weights of salmon at the end of the simulated season (Fig. 8) correspond to the drop in food availability in the top 20 m (Fig. 1). Most likely, these fish actually change dramatically in their feeding habits by this late period in the year, and our model becomes unrealistic during this period. However, since this occurs after the fish have attained relatively large body size, it has little effect upon survival. Food preferences and searching methods may change during this time and should be investigated.

CRITICAL ASSUMPTIONS AND DATA NEEDS

There are two major problems in the feeding and growth model described here. The first is the method of formulation: should it be based upon searching (Eggers 1977) or the ration method currently used? A major ingredient of this problem is the measurement of effective food concentration. Laboratory feeding experiments (Parsons and LeBrasseur 1970) show little feeding occurring at the concentrations found in the ocean. We assumed that patchiness concentrates food for salmon. It is critically important to understand the mechanism of salmonid searching and feeding to understand when food might become limiting in spite of the ability of the fish to find patches. For example, as salmon density increases, the distance between food patches might also increase as more patches are exploited or there could be interference effects as fish become more crowded within patches. All of our predictions about reduced growth leading to reduced survival depend upon the point at which the fish can no longer get a maximum ration, and our estimation of that point is contingent upon a tenuous set of assumptions.

The second major problem in this model is the appearance of low growth at the end of the year. These fish are probably switching to large food items by this point, but within the context of the current synthesis effort we did not attempt to model this change. Few data exist other than measures of gut contents (Manzer 1969). We feel this is another area of great importance. Ideally a study could be executed that determined the searching, prey selection, prey handling, and ingestion of food items. The technical problems would be substantial, but the benefits would be great.

Seaward and Coastal Migration of Young Salmon

The model of the migration of young salmon consists of three major parts, beginning with the seaward migration of fish into the marine environment. Each of the 10 blocks of fish (Table 2) is broken into 10 subblocks, each constituting 3 days worth of migrants. Numbers, average weight, and location are carried in the model for each of these subblocks. The estimated mean time of arrival at the sea is placed at the center of the 30-day period over which any particular block enters the sea, and the total number of migrants is allocated over the arrival time in a normal distribution with an appropriate standard deviation.

Blocks are allocated geographically as follows: all five species from the south coast, Strait of Georgia area enter in the most southerly spatial strip of the model. This is intended to represent the Fraser River stocks.

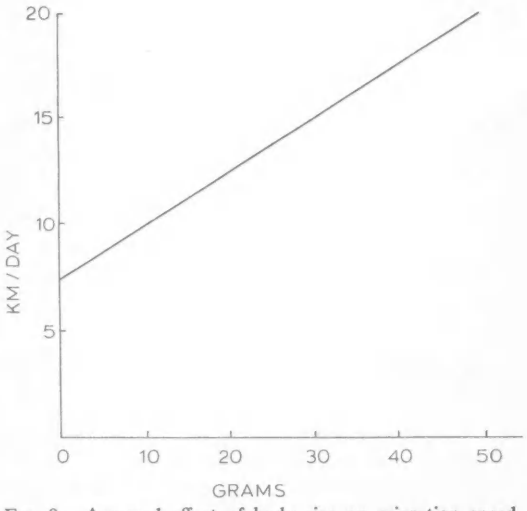

FIG. 9. Assumed effect of body size on migration speed.

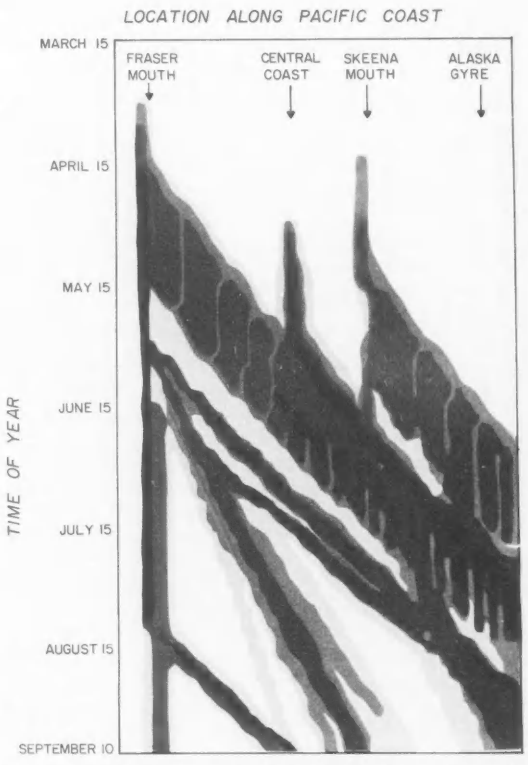

FIG. 10. Simulated space–time distribution of juvenile salmon along British Columbia coast. Dark areas show regions with large salmon abundances at the indicated time.

Blocks of pink and chum salmon enter the model in the midcoast region, representing the Johnstone Strait area. Blocks of sockeye, coho, and chinook salmon enter the model from the Skeena River. Due to computational constraints upon the numbers of blocks that can be efficiently handled, Skeena pink and chum are not represented explicitly. Instead, the pink and chum blocks entering the model in the Johnstone Strait area are increased to represent the Skeena stocks of these species.

The second part of the model represents residence of fish immediately after they have entered the marine environment. In the model, fish remain in the area of first entry for various lengths of time. For example, the Fraser pinks are believed to spend up to 4 mo in the Gulf Islands before beginning further migration (D. Blackbourn personal communication, see Table 1). On the other hand, sockeye salmon are believed to begin migration as soon as they enter the sea.

The final part of the migration model is the movement of fish from their first ocean residence. We assume that the fish always move northward and remain in narrow bands along the coast of British Columbia and the Alaska panhandle. Though little is known about the early migration of salmon, there is some evidence to support this assumption (French et al. 1976). The speed of migration is assumed to depend only upon the size of the fish, and we used an estimated relationship (Fig. 9) based on the known length of time it takes a fish to move up the coast (Royce et al. 1968) and the effect of body size on swimming speed.

Data used for the nominal conditions of the model (Table 2) were developed through discussions among the workshop participants, and only represent a rough characterization of current conditions. They do not represent year-to-year variation in run sizes or timing. For example, the data represent a "pink year" rather than a "sockeye year" on the Fraser River. For the purpose of an initial overview these data are adequate; but if the model were to be used for detailed predictions, more accurate information would be necessary. In the following section, the effect of changing some of these data on model predictions will be discussed.

RESULTS FROM THE MIGRATION MODEL

Predictions from the migration model come in two forms. First, we generate a time–space map of juvenile fish distribution along the coast (Fig. 10). Such maps could be an important tool in planning where and when to enhance salmon. If there is any possibility of competition for food between enhanced and natural stocks, then it would be prudent to reduce interactions along the coast by appropriate timing and siting of enhancement facilities. Alternatively, if natural stocks actually do avoid certain places at certain times, as our calculations indicate, then there may be good biological

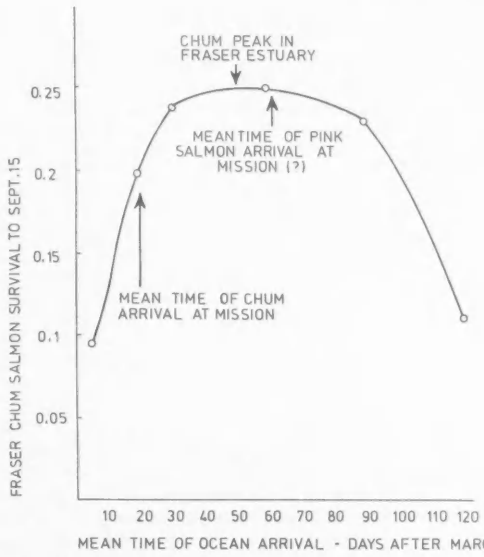
CHUM PEAK IN FRASER ESTUARY
MEAN TIME OF PINK SALMON ARRIVAL AT MISSION (?)
MEAN TIME OF CHUM ARRIVAL AT MISSION
FRASER CHUM SALMON SURVIVAL TO SEPT. 15
0.25
0.2
0.15
0.1
0.05
10 20 30 40 50 60 70 80 90 100 110 120
MEAN TIME OF OCEAN ARRIVAL - DAYS AFTER MARCH 15

FIG. 11. Simulated effect of arrival timing on Fraser chum salmon survival.

reasons why enhanced stocks that are produced to occupy these underutilized spots in space and time will be prone to low survival.

The second type of prediction from the model was obtained by altering functional components and examining the effect on predicted survival rates of the various stocks (see next section for survival processes). Alterations were done for two reasons. First, we wanted to reflect changes that may occur as a result of enhancement. Second, we wanted to determine the behavior of the model under various assumptions, since there is and will likely continue to be a great deal of uncertainty about the details of space–time distribution.

The time of seaward migration of some of the stocks is not well known. For example, there is uncertainty about the length of time Fraser River chums spend in the river delta marshes before heading out to sea. Figure 11 shows predicted survival rate of chum salmon through their coastal migration as a function of the mean time of seaward migration. The peak predicted survival occurs a considerable time after the fish are counted at Mission, B.C., which is upstream from the Fraser estuary. Therefore if the model is correct, the fish should remain in freshwater below Mission until conditions are best in the salt water. Studies have shown that chum salmon are present in the Fraser delta marshes for up to 1 mo after passing Mission (Dunford 1975); a similar pattern has been found in the smaller Nanaimo River estuary (Sibert et al. 1977).

The existence of this special life history feature (estuarine holding), which the model predicts should exist, lends credibility to assertions that the model does represent at least some of the main biological problems actually faced by the fish. In the model, lower chum survival given very early or late migration into open water is due to lower food supplies (surface plankton) leading to lower growth and longer time at risk to size selective mortality.

TABLE 4. Simulated effect of residence duration on pink and chum salmon survival.

Survival to Sept. 15	Duration of residence of Fraser pink salmon in first ocean area				
block	0	1 mo	2 mo	3 mo	4 mo
Pink	.12	.11	.11	.10	.10
Chum	.11	.12	.11	.10	.10

TABLE 5. Simulated effect of chum and pink salmon separation in early ocean residence.

	Survival to Sept. 15	
Block	Remain in same area	Separated in first residence area
Fraser pink	.10	.11
Fraser chum	.10	.11

There is uncertainty about the duration of residence in local waters off the mouth of rivers. This is likely the time of greatest mortality, and it may also be very important in the growth of the young salmon. Results from the simulation show that a decreased residence time for Fraser pinks below the observed four months results in increased survival (Table 4). This result leads one to suspect that the model does not represent some very important processes that occur during the several months that pinks spend in the Gulf of Georgia.

Another weak assumption about local residence is that all the stocks from each river system reside in the same place. When the pink and chum salmon from the Fraser River are separated in space (chums bypass first coastal strip), the model predicts that survival of both stocks should increase slightly (Table 5). This result is encouraging for it is believed that these stocks may in fact be separated in their first ocean residence (D. Blackbourn personal communication).

Predictions are critically sensitive to the width and thickness of the coastal band within which migration occurs (Table 6). Increasing the width implies greater total food supplies. Increasing the depth implies a more complex interplay between fish blocks, since any block can more completely deplete the food supply per unit area as it passes (see following subsection).

A final issue with respect to the assumptions in the migration model is the movement rate. The crude guess incorporated in the model has very little data to substantiate it. Workshop participants suggested that migration rate may be affected by the availability of food in

TABLE 6. Proportion surviving to September 15 of all blocks for various levels of enhancement of Fraser pinks under different assumptions of feeding depth and width of migration band along the coast.

Block	Feeding depth 2 m 37.5 km wide strips Fraser pinks at					Feeding depth 20 m 37.5 km wide strips Fraser pinks at					Feeding depth 20 m 5 km wide strips
	Normal	2x	4x	8x	10x	Normal	2x	4x	8x	10x	Fraser pinks normal
Fraser sockeye	.45	.45	.45	.45	.45	.45	.45	.45	.45	.45	.45
Skeena sockeye	.48	.48	.48	.48	.48	.48	.48	.48	.48	.48	.48
Fraser pink	.10	.09	.07	.04	.03	.10	.09	.08	.04	.03	.04
Central coast pink	.19	.19	.19	.19	.19	.19	.19	.19	.19	.19	.16
Fraser chum	.11	.10	.09	.08	.07	.10	.10	.09	.07	.06	.07
Central coast chums	.19	.19	.19	.19	.19	.18	.18	.18	.18	.18	.17
Fraser coho	.61	.61	.59	.56	.54	.59	.58	.56	.52	.50	.53
Skeena coho	.60	.60	.60	.60	.60	.59	.59	.59	.59	.59	.60
Fraser chinook	.10	.10	.09	.08	.08	.10	.10	.09	.07	.06	.07
Skeena chinook	.33	.33	.33	.33	.33	.32	.32	.32	.32	.32	.33

localized areas. While the feeding submodel attempts to represent very short-term effects of this type through a patchiness factor, the longer term effect of a patchy food source on migration speed is not known. Thus we tried simulations with different values for the slope of migration speed vs. body size relationship. The conclusion was that the slower the movement, the lower the survival (Table 7). However, there is so little known about this important component that it is difficult to determine bounds of uncertainty or for that matter, whether migration speed has anything to do with body size.

Studies of the migration of small fish are very difficult. However, it will be impossible to predict more accurately the effects of an enhanced stock of salmon without information on where and when the fish are during their first few months at sea.

Natural Mortality

Several mortality agents may affect juvenile salmon in the ocean, including predators, disease, parasites, and starvation. The mortality rate due to each agent is probably related to density of salmon, but the paucity of existing data prevents even educated guesses at what these relationships are. For example, the rate of spread of some disease through a population may be an exponential function of density, but the initial incidence of the disease may be a completely unpredictable random factor. Similarly, predation can be substantial from seals, birds, or other fish, each of which has a different response to density of salmon, and for which there are virtually no data.

It was decided that many of these mortality agents could be implicitly represented by calculating mortality rate as a function of body size, with larger fish having lower mortality rates. This assumes that the effect of all mortality agents will be mediated by body size, with larger fish subjected to fewer classes of mortality. While data substantiate this relationship for some, but not all salmon stocks, the data describe the relation only over the whole ocean life of the stocks; there is no comparable information for the early period we are modeling. The ocean mortality data reviewed by Ricker (1976) provide some evidence to estimate the parameters of the relationship, though body size estimates at the corresponding times are lacking. The relation used in the model is:

$$P = A \cdot e^{-BW} \quad (1)$$

where P = proportion of the salmon stock dying each 3-day time period, A = a parameter (.3), B = a parameter (.15), and W = body weight of fish in grams. Since there is no evidence to the contrary, the model assumes that all species-location blocks are subject to the same mortality rate–body size relationship.

TABLE 7. Survivals for all stocks at various migration speed assumptions.

Block	½ Normal	Normal	2x Normal
Fraser sockeye	.42	.45	.48
Skeena sockeye	.43	.48	.56
Fraser pink	.10	.10	.10
Central coast pink	.16	.19	.21
Fraser chum	.10	.10	.11
Central coast chum	.15	.18	.21
Fraser coho	.58	.59	.59
Skeena coho	.53	.59	.67
Fraser chinook	.10	.10	.11
Skeena chinook	.29	.32	.35

PREDICTIONS

Table 6 shows simulated total survival rates of the 10 different blocks over the 200-d period. Note that the relative survival rates of the different species seem about right; pinks and chums are about the same and have the lowest survivals, while coho have the highest survivals. One parameter in the mortality–body size relation was altered to test its sensitivity, and results are shown in Table 8. Survivals are substantially lower but

TABLE 8. Sensitivity of survival estimates to changes in mortality rate vs. body size assumption. B is the density effect parameter of equation (1) in the text.

Block	Predicted survival until Sept. 15 with $B = 0.15$	Predicted survival until Sept. 15 with $B = 0.1$
Fraser sockeye	.45	.20
Skeena sockeye	.48	.27
Fraser pink	.10	.03
Central coast pink	.19	.08
Fraser chum	.10	.03
Central coast chums	.18	.07
Fraser coho	.59	.25
Skeena coho	.59	.26
Fraser chinook	.10	.02
Skeena chinook	.32	.13

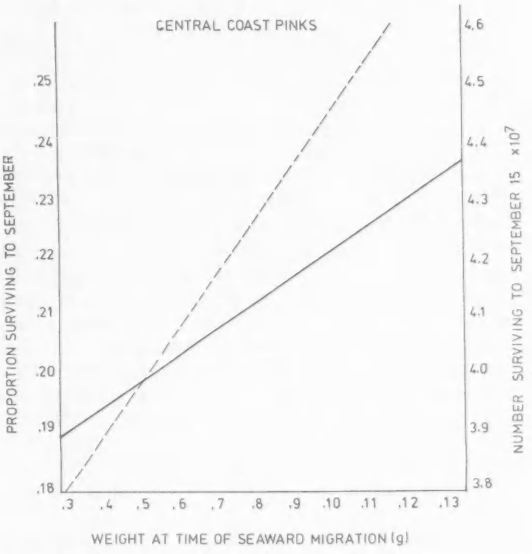

FIG. 12. Simulated effect of increased fry size on pink salmon survival; dashed line is number surviving, solid line is proportion.

the relative survivals of different species remain the same.

One form of enhancement being contemplated in British Columbia is to hold pink fry and prefeed them to a larger body size before release. We altered the body size of migrating fry for Fraser and central coast pinks and got similar results for these two stocks (Fig. 12). When mean body size at entry into salt water is raised above the present 0.32 g, survival increases linearly, though not very much. However, when one looks at the increase in numbers resulting from, say, tripling the body size, large increases in total returning adults appear to be possible.

It is also possible to alter the downstream run timing of a stock, by narrowing or spreading times of release

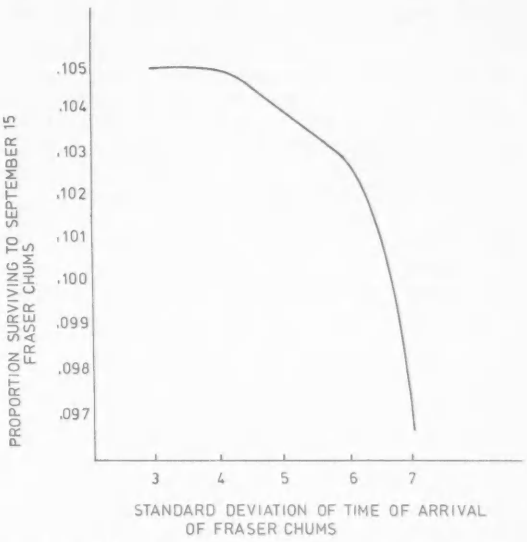

FIG. 13. Simulated effect of changing the distribution of time of Fraser chum salmon ocean arrival.

from enhancement facilities. When the spread of Fraser chum run timing was modified, there was some alteration in survival of that stock (Fig. 13). Survival increased when the run timing distribution was narrowed, and decreased when it was broadened. The explanation of this is made clearer by referring to Fig. 11, which shows the predicted relationship between Fraser chum survival and mean run timing. Since Fraser chums enter salt water around the time that will result in peak possible survival, a larger proportion of the population enters the ocean during the higher survival period when the run timing distribution is narrowed. In contrast, when the run is more spread out, more chums migrate too early to survive well.

The number of fish entering the ocean from various blocks was altered to test for any significant density effect upon survival. Table 6 is an example of the model's output for alteration to the Fraser pink block. Here survival for all blocks is shown in relation to various fry outputs of Fraser pinks, from 200 million to 2 billion, under different assumptions about the depth range where feeding occurs. The results indicate that there is a density effect upon survival of the pinks, and that the other Fraser blocks, with the exception of sockeye, are also adversely affected. The effect that the pinks have upon the other species is increased when it is assumed that salmon feed down to 20 m instead of just 2 m. At a shallow feeding depth, the increased number of pink salmon does not have as large an effect upon the total food supply as at a deeper feeding depth. Therefore the surface food supply can recover faster and decreases the impact of pink salmon upon the other blocks of fish.

Critical Assumptions and Data Needs

Overall model predictions are quite sensitive to the mortality vs. body size relation. This is especially true for conditions where there is severe competition for food among salmon, because the resulting slower growth will significantly increase the mortality rate and thereby rapidly decrease the competitive effect. This feedback relation is thus a critical one which deserves further study. Also, it should be determined if different species have the same underlying mortality–body size relationship.

Virtually nothing is known about the relation between mortality rate and marine salmon density. In particular, predator functional and numerical responses could have a profound effect on increasing densities of salmon, as in freshwater (Peterman and Gatto 1978). While we recognize the logistical difficulties of measuring these responses of predators in the ocean, it seems imperative to learn something about them, because it is possible that increasing numbers of fry or smolts will not saturate predators; mortality rates could increase to compensate the increased numbers of juveniles. There has been a preoccupation in recent field studies on the trophic basis for salmon production (food, feeding, and growth); our results suggest that the emphasis should be shifted up one level, to a much more careful look at the link between salmon and higher predators.

Discussion

Though we have tried to evaluate potential problems faced by juvenile salmon in some space–time detail, the model proposed above basically represents a very simple hypothesis: mortality rates decrease with increasing body size, and any density-related mechanisms that produce slower growth will ultimately result in higher cumulative mortalities. This is not a new hypothesis for salmon (e.g. Henry 1961), and related models based on the direct dependence of mortality on growth rate (rather than resulting body size) have been proposed for pelagic marine fishes (Jones 1973; Jones and Hall 1974; Ware 1975). Models based on size- or growth-dependent mortality generally make two predictions that may not be intuitively obvious: (1) large density-dependent mortality may occur without large accompanying changes in standing crops of available food, since small decreases in food supply can rapidly feed back through the mortality to produce lower cropping pressure; and (2) growth rates measured on surviving fish need not reflect competitive situations that the stock has faced earlier, since slower growing individuals will have been lost and thus will not be included in the measurement.

This study has emphasized trophic relationships as potential limits to salmon enhancement. We felt that while the rearing capacity of the Pacific Ocean as a whole may be effectively unlimited, a model might help uncover some more subtle problems in terms of the seasonal and spatial arrangement of juvenile fish and their food supplies. Such problems have not emerged; apparently there is enough food production to support several times the existing abundance of juvenile salmon without noticeable effects on growth and size-related survival, even assuming that the useable coastal zone is very narrow and considering spatial position effects within large migrating blocks of fish. Existing food supply data are inadequate to test this conclusion; one bit of supporting evidence is that Gulf of Georgia zooplankton biomasses were not lower in 1966 and 1968, when pink salmon juveniles were present, than in 1967 when pink salmon were absent (Stephens et al. 1969).

We must look elsewhere for explanations of observed density dependence of marine survival rates in stocks like the Skeena sockeye. Possibilities that we have not explored include: (1) special restrictive features of some coastal areas such as narrow inlets; (2) density-dependent predation (some predators may not turn to salmon until the salmon become abundant); (3) interference competition involving direct behavioral effects of individuals on one another when densities are high, even if the food supply is not heavily exploited; and (4) mechanisms operating during downstream migration, estuarine residence, or later in ocean life.

However, we have not been able to resolve some key uncertainties about trophic relationships: (1) how fast is the available (near surface) food supply replenished by vertical dispersal or migration of food organisms? (2) how efficient are juvenile fish at finding food when it becomes even slightly more scarce, or how much would food abundance have to be reduced before growth effects would become noticeable? (3) are juvenile salmon strongly restricted to nearshore environments, or would they simply (and safely) move farther offshore in the face of increased competition? (4) can mortality rate be predicted only from body size, or must other measures of physiological state, species, location, and seasonal timing also be considered?

These uncertainties should not be considered a condemnation of salmon enhancement programs. We have been digging for weak points, and it is not surprising that we have found some. If questions like we have raised are considered in the design of studies to monitor enhancement as it proceeds, there is enormous potential for learning more about salmon. The alternative is to be caught without any answers when the inevitable difficulties do arise.

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Natural Convection: A Mechanism for Transporting Oxygen to Incubating Salmon Eggs

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Respiratory exchange at the surface of developing fish eggs is influenced by two processes: diffusion and convection. The respiring egg acts as an oxygen sink, removing dissolved oxygen from the diffusion layer surrounding the outer surfaces of the egg capsule. To maintain the oxygen gradient in the diffusion layer, oxygen must be delivered to it by convection. This paper describes two components of convective transport: that provided by *forced* convection — the bulk transport of oxygenated water to the egg; and a newly recognized component, *natural* convection. Salmon normally spawn in gravel environments. Mass transfer of oxygen to incubating eggs, resulting from forced convection, is provided by subsurface water moving with a velocity related primarily to properties of the gravel and the hydraulic gradient. Egg-induced natural convection is seen as a component embedded in these relations. Acting as an emergency oxygen transport and metabolite disposal mechanism at low water velocities, natural convection could maintain perfusion of respiring eggs at some minimum water velocity in the event of diminution or interruption of natural water flow. The relevance of natural convection as a possible mechanism affecting several other respiring systems is discussed.

Key words: respiration, forced convection, density of water, bulk transport, diffusion, diffusion layer, oxygen gradient

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Deux processus influent sur les échanges respiratoires à la surface d'oeufs de poisson en voie de développement : la diffusion et la convection. L'oeuf qui respire agit comme un piège à oxygène, enlevant l'oxygène dissout de la couche de diffusion qui entoure les surfaces extérieures de la capsule de l'oeuf. Pour que le gradient d'oxygène se maintienne dans la couche de diffusion, il lui faut recevoir de l'oxygène par convection. Nous décrivons dans cet article deux composants du transport convectif : la convection *forcée* — le transport en masse d'eau oxygénée vers l'oeuf et un composant récemment reconnu, la convection *naturelle*. Les saumons frayent normalement sur des substrats de gravier. Le transport en masse de l'oxygène aux oeufs en voie d'incubation, résultant de la convection forcée, est fourni par l'eau de la sous-surface se déplaçant à une vitesse qui dépend surtout des propriétés du gravier et du gradient hydraulique. Nous considérons la convection naturelle provoquée par les oeufs comme un composant inhérent à ces relations. La convection naturelle agit comme un mécanisme de transport d'urgence de l'oxygène et de rejet des métabolites à de faibles vitesses de l'eau, et elle peut ainsi maintenir la perfusion des oeufs qui respirent à une vitesse minimum de l'eau dans le cas d'une diminution ou d'une interruption du débit naturel. Nous examinons la convection naturelle comme mécanisme possible affectant plusieurs autres systèmes qui respirent.

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DISSOLVED oxygen is provided to the living teleost egg by two processes: diffusion and convection. The first of these is a statistical molecular process that proceeds toward equilibrium between two regions of differing oxygen concentration. The respiring egg, acting as an oxygen sink, removes oxygen from the perivitelline fluid beneath the egg capsule, thereby establishing an active

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concentration gradient between the interior of the egg and the external microenvironment surrounding the egg. In that microenvironment, metabolic activities of the egg — oxygen consumption and metabolite excretion — create a concentration gradient between the capsule and the macroenvironment, known as the diffusion layer. To maintain the gradient, oxygen must be supplied continually to the diffusion layer. This is provided by convection, the second process, which is a bulk movement of the water perfusing the egg. In general, transport of oxygen to the diffusion layer depends primarily on convective velocity and the concentration of oxygen in the convected water.

Until recently, it was assumed that supply of oxygen to the diffusion layer was dependent entirely on *forced* convection (Daykin 1965) — bulk movement of water perfusing the egg in relation to properties of the surrounding gravel and the hydraulic gradient. We now find that another component, *natural* convection, also provides oxygen to the diffusion layer. We have observed this in coho salmon (*Oncorhynchus kisutch*) eggs near hatching, respiring in air-saturated water. Natural convection results from density differences produced at the egg surface, through removal of dissolved oxygen from and excretion of metabolite into the diffusion layer. Both of these activities result in an increase in density of the water in the macroenvironment. Natural convection, as well as the causes of the density increase that promote it, to our knowledge have been unsuspected in their potential influence on the function of respiratory surfaces.

Natural convection itself is a well-documented phenomenon, especially in the electrochemical behavior of vertical electrodes (Wagner 1949; Wilke et al. 1953; O'Brien et al. 1963; O'Brien and Mukherjee 1964; O'Brien 1972). Convection due to removal of dissolved oxygen is less well known; only recently has it been shown that oxygen solutions are less dense than pure water (O'Brien and Hyslop 1975). They found that perturbations to a vertical fringe system were produced when the pressure of oxygen was raised over water in an interferometer. The perturbed fringes could be fitted by the solution of Fick's Second Law of Diffusion with the appropriate boundary conditions. That is

$$(1) \qquad C_{(x,t)} = (C - C_o)\,\mathrm{erfc}\,(x/(2(Dt)^{\frac{1}{2}})) + C_o$$

where $C_{(x,\,t)}$ is the concentration of oxygen ($\mathrm{mol \cdot cm^{-3}}$) at a distance x (cm) perpendicular to the water surface at time t(s), C_o is the bulk or zero concentration, erfc is the error function complement, and D is the diffusion constant of oxygen ($\mathrm{cm^2 \cdot s^{-1}}$). Both theory and experience with fringes perturbed by convection (O'Brien 1972) indicate that the observed fringe pattern perturbations were not caused by convection. Experiments show that there is a negative slope for the relation between refractive index and concentration (or pressure) (O'Brien and Hyslop 1975). This is consistent with the fact that a decrease in density would be coupled with increased concentration or pressure, as seen in the Lorentz–Lorenz expression

$$(2) \qquad R = M/\rho((n^2 - 1)/(n^2 + 2))$$

where R is the molar refraction ($\mathrm{cm^3}$), M is the molecular weight of the solute (g), n is the measured refractive index of the solution, and ρ is the density ($\mathrm{g \cdot cm^{-3}}$). Since R and M are constants, it follows that if n decreases with concentration, then so must ρ.

On the other hand, $(NH_4)_2CO_3$ can be assumed to result from the metabolic products

$$(3) \quad 2NH_{3(aq)} + CO_{2(aq)} + H_2O \rightleftharpoons (NH_4)_2CO_3 \rightarrow 2NH_4{}^+{}_{(aq)} + CO_3{}^{2-}{}_{(aq)}.$$

Experiments have shown that solid $(NH_4)_2CO_3$ and its solutions are heavier than water (unpublished data, O'Brien 1978). We also have found carbon dioxide solutions to be heavier than water. We have not conducted experiments on the density of NH_3 solutions; however, they are listed (Handbook of Chemistry and Physics, Chemical Rubber Publ. Co., 1958; p. 1963) as being less dense than water. It is assumed that the CO_2 and NH_3 produced during egg development originate largely from protein metabolism; apart from water, protein is the dominant constituent of the salmon egg (Hayes 1949). Since the C/N ratio in protein can be expected to be at least 10:1, it seems likely that CO_2 will be present in greater quantity than NH_3. That being the case, the equilibrium in equation 3 will be far to the right, and no light ammonia solution is likely to be generated to oppose downward convection. Certainly no visible effects contrary to this assumption have been detected. Therefore, both processes would contribute to and be responsible for the observed convection. Further, it appears that oxygen uptake by the egg is responsible for the initial convective propelling force over short experimental elapsed times (0–5 min), and the excretion of metabolites is the main continuing convective force.

Under normal conditions the rate of oxygen consumption of salmon eggs increases, as embryonic development proceeds, and reaches a maximum just prior to hatching. As with the eggs of most species of Pacific salmon, those of the coho hatch after an incubation period of about 50 d at 10°C (500 degree-days). The coho eggs used in these studies were between 450 and 500 degree-days old, and hatching did occur during experiments in some of the later tests. The rate of oxygen uptake of salmonid eggs just prior to hatching varies with temperature, and ranges from about 1.5×10^{-3} to 5.5×10^{-3} mg/egg per h (Hayes et al. 1951; Wickett 1954; Alderdice et al. 1958; Marckmann 1958; J. O. T. Jensen, Pacific Biological Station, personal communication). Rates of oxygen uptake of the coho eggs in the current tests at 10°C would be expected to be about 3×10^{-3} to 5×10^{-3} mg/egg per h.

This paper demonstrates natural convection. It shows that the process, embedded in forced convective transport, could provide a substantial portion of the total

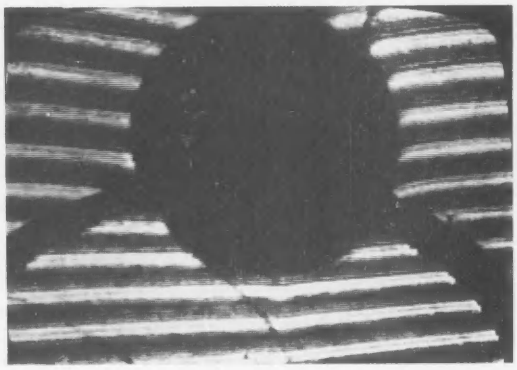

FIG. 1. Interferogram of coho salmon eggs near hatching. Three eggs, in a row perpendicular to the plane of the paper, are held in a thin, open-sided holder, which appears as a dark, curved line on each side of the eggs. The interference fringes bend at the egg surface, indicative of a concentration change in that region. There also is a slight discontinuity in the fringes immediately below the eggs; the series of "dips" in the fringes result from downward convective streaming of water in the diffusion layer at the egg surface to a point at the bottom of the eggs. There the layer breaks free and flows vertically away from the eggs.

respiratory requirements of incubating salmon eggs under circumstances where forced convection suffers either brief interruptions, or reductions due to decreased bulk water movement over longer periods. The process also helps to explain differences between bulk transport requirements estimated on the basis of forced convection mass transfer of oxygen (Daykin 1965; Wickett 1975), and those presumed lower requirements sometimes reported in practical incubation situations (Bams and Simpson 1977).

Materials and Methods

Coho salmon eggs obtained from Rosewall Creek, Vancouver Island, on November 30, 1976, were fertilized and incubated at 6°C at the Pacific Biological Station, Nanaimo. When the eggs were about 400 degree-days old, and well eyed, they were transported to the University of Victoria, Department of Chemistry, and held at 8°C until needed.

Two types of experiments were conducted: interferometry, and measurement of particle movement in the water containing the eggs in the interferometer. For each experimental run, three eggs were first washed in terramycin solution (50 mg/L for 5 min at 8°C) to remove bacterial contamination, then rinsed in water at 8°C. Next, the eggs were placed in an open-sided aluminum rack inserted lengthwise into a thermostated (10°C) Fabry-Perot interferometer, similar in design to that described by O'Brien and Hyslop (1975). The interferometer then was carefully filled with air-saturated water at 10°C.

The first type of experiment, interferometric measurement, used 90% reflecting optically flat surfaces for the ends of the interferometer cell and a laser source emitting coherent light at 632.8 nm to produce interference fringes. Perturbations to the fringes developing at the egg surfaces (Fig. 1) are related to changes in the concentration of solutes involved in respiration and metabolite excretion. The second type of experiment measured the direction and velocity of particle movement in the water in the interferometer. In this instance, clear end windows replaced the reflecting surfaces on the ends of the interferometer cell, and small particles of Plexiglas® (<0.1 mm diam) were suspended in the air-saturated water. Movement of the particles, denoting convection, was readily observed and photographed (Fig. 2A).

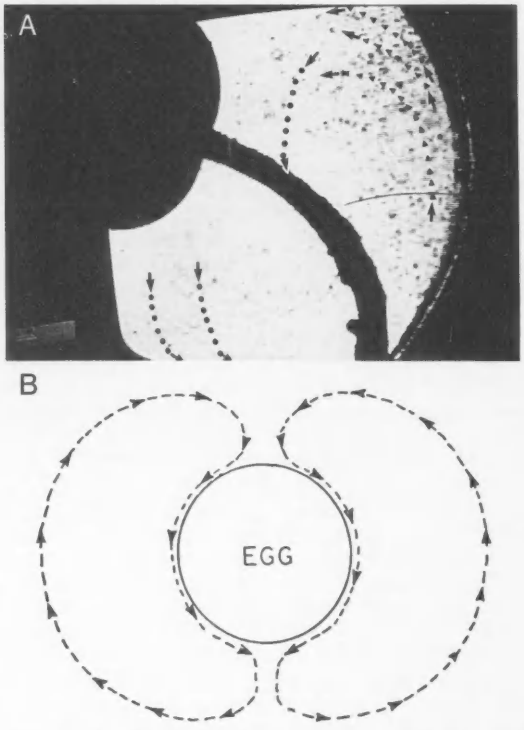

FIG. 2. A, Photograph of coho eggs, as in Fig. 1, made using clear end windows in the interferometer cell. The paths of six fine particles of plastic suspended in the water surrounding the eggs are shown in composite from series of photographs taken consecutively. The arrows indicate the direction of movement. B, Stylized depiction of toroidal motion of water surrounding a respiring egg under the influence of natural convection in a "static" environment without substrate or constraining boundaries. The movement illustrated would be altered substantially by the presence of forced convection (bulk movement of water) and the characteristics of natural substrate (gravel) surrounding the egg.

Results

The configuration in Fig. 1 shows a typical interference fringe pattern 5 min after the beginning of a test. The bends in the fringes at the egg surface indicate the presence of a larger concentration gradient and, con-

TABLE 1. Typical velocities of Plexiglas ® particles (<0.1 mm diam) suspended in water surrounding coho salmon eggs during natural convection.

Particle no.	Time observed (s)	Velocity ($cm \cdot s^{-1}$)
1	9	0.026
2	7	0.021
3	21	0.019
4	24	0.014
5	12	0.015
6	28	0.017
7	18	0.024
8	13	0.022
Avg (unweighted)		0.020

sequently, higher convective velocities. In that region of increased gradient, the diffusion layer decreases in thickness. The denser solution in the gradient flows down over the surface of the eggs to converge at the bottom of the eggs into a rapidly downflowing stream, indicated by the "dip" in the fringes directly beneath the eggs.

Figure 2A shows successive positions of a series of particles photographed within the cylindrical interferometer cell with the eggs placed axially. Figure 2B is a stylized interpretation of the toroidal motion that might be expected in the absence of the cylindrical walls of the interferometer, and without substrate particles (e.g. gravel) surrounding the egg. This configuration, of course, would be greatly modified under natural conditions. Typical measured particle velocities (Table 1) averaged about 0.020 $cm \cdot s^{-1}$ (72 cm/h). In a control experiment where teflon balls of the same size replaced the eggs, neither perturbation to the fringes nor convection of the suspended particles occurred.

Discussion

In diffusion and convection studies, the general form of the transport equation for uncharged particles is

$$\vec{J} = \vec{J}_D + \vec{J}_C = -D\frac{\partial c}{\partial x} + cV \tag{4}$$

where $\vec{J}_D$ and $\vec{J}_C$ are the diffusion and convection components, respectively, of the total dissolved oxygen flux $\vec{J}$ ($mol \cdot cm^{-2} \cdot s^{-1}$), D is the diffusion coefficient for oxygen in water ($cm^2 \cdot s^{-1}$), c is concentration of dissolved oxygen in the bulk fluid ($mol \cdot cm^{-3}$), x is the distance from the surface of the egg capsule (cm), and V is the bulk velocity of the perfusing water ($cm \cdot s^{-1}$). The configuration of our cell necessitates using a factor of 0.5 for the convective term: as approximately half of the water in the interferometer cell appears to be rising, judged on the basis of particle movement, then the other half must be sinking.

The most acceptable value of the diffusion coefficient for oxygen in water is 2.0×10^{-5} $cm^2 \cdot s^{-1}$ at 22°C (Tham et al. 1970; O'Brien and Hyslop 1975). This value, converted to 10°C by the method of Davis et al. (1967), yields $D = 1.4 \times 10^{-5}$ $cm^2 \cdot s^{-1}$. Also, at 10°C the concentration of oxygen in air-saturated freshwater is 0.35 $\mu mol \cdot cm^{-3}$ (11.2 ppm) (Washburn et al. 1928). With these data we may compare the relative effectiveness of diffusion and convection as mechanisms for transfer of oxygen to the respiring eggs.

In assessing the role of natural convection as a mechanism for oxygen transport, it is necessary to estimate the concentration of oxygen in the microenvironment surrounding the capsule of the respiring egg. Cooper (1965) examined the relation between survival of sockeye salmon (*O. nerka*) eggs in a gravel medium and the bulk velocity (discharge per unit area of gravel, eggs, and water) of water perfusing the eggs. Wickett (1975, table 1) applied Daykin's (1965) theory of mass transfer to Cooper's results. Interpolating Wickett's tabled values, we assume that the minimum oxygen concentration at the egg surface — the interior boundary of the diffusion layer — is about 0.334 $\mu mol \cdot cm^{-3}$ (10.7 ppm) at a bulk velocity of 0.020 $cm \cdot s^{-1}$ (Table 1), where the concentration of oxygen in the macroenvironment (Wickett 1975) was 0.39 $\mu mol \cdot cm^{-3}$ (12.5 ppm). In the present instance, using a measured diffusion layer thickness of 0.035 cm, we calculate $\vec{J}_D$ to be no more than 6.4×10^{-6} $mol \cdot cm^{-2} \cdot s^{-1}$. For convection, with $V = 0.020$ $cm \cdot s^{-1}$, $\vec{J}_C$ is calculated to be 3.5×10^{-3} $mol \cdot cm^{-2} \cdot s^{-1}$ of dissolved oxygen. It is seen that $\vec{J}_C/\vec{J}_D = 5.5 \times 10^2$. Therefore, convection is estimated to be about 500 times more effective than diffusion in providing oxygen to the respiring egg in our "static" water experiment. The actual mechanism of increased transport, of course, is the reduction by natural convection of the thickness of the diffusion layer — the diffusion distance.

Furthermore, the magnitude of perturbation of the fringes (Fig. 1) cannot be accounted for solely on the basis of oxygen depletion in the diffusion layer. We conclude that the remainder of the refractive index (concentration) change is a result of the presence of metabolites, which also must contribute to convection. From these considerations, $\vec{J}$ must be in the range of $3\text{–}4 \times 10^{-3}$ $\mu mol \cdot cm^{-2} \cdot s^{-1}$.

Our interferometer cell is designed so that convection, if present, is optimized. For salmon eggs respiring in a gravel substrate, the less symmetrical environment could reduce the convective mode by an order of magnitude. The overall effect, we believe, is that convection in a natural situation may result in a rate of oxygen transport at least one order of magnitude greater than that associated with diffusion. Of course, our results serve primarily to indicate the potential value of natural convection. A fuller evaluation of the phenomenon must await further analyses, particularly for eggs at different temperatures, stages of embryonic development, and at various environmental levels of dissolved oxygen.

Natural convection as an oxygen transport mechanism is different from, and would be embedded in, the

forced convection of mass transfer as treated by Daykin (1965). Bams and Simpson (1977), on the basis of available evidence, suggest that forced transport of water past incubating eggs under practical conditions should be about 200 cm/h, with a minimum permissible limit of 150 cm/h. Nevertheless, these authors mention that satisfactory results appear to have been obtained at lower bulk velocities in some instances. At these lower bulk velocities it now appears that natural convection may assume a significant role in oxygen transport. However, at this time natural convection alone should be considered realistically only as an emergency system, providing oxygen to respiring eggs in the event of an interruption or diminution in bulk flow. Natural convection can oxygenate eggs only within the limited macroenvironment surrounding the egg that is subject to convective transport. If oxygen in the macroenvironment were not replenished — either in our interferometer cell or under natural conditions — then succeeding convective cycles (e.g. Fig. 2B) would slowly deplete dissolved oxygen in the limited water volume presented to the egg, and the egg eventually would die from lack of oxygen.

In general, natural convection is seen as a mechanism that could supply a substantial portion of oxygen requirements to stationary wetted vertical or near-vertical respiring surfaces. Hence, we conclude that oxygen-absorbing surfaces are likely to be predominantly vertical or inclined in all stationary aquatic organisms. Natural convection may be involved in the supply of oxygen to various tissues hitherto thought to depend primarily on diffusion. These may include the osteocytes in the extravascular canalicules of the Haversian system of compact bone, tissues such as those surrounding the fluid-filled synovial capsule of diarthroidal joints (e.g. knee joint), and some types of connective tissues. Possible applications of natural convection might be found in methods of manipulating and treating such tissues following disease or injury.

Natural convection may also be associated with oxygenation of eggs in the interior of egg masses laid on fixed substrate. For example, the Pacific herring (*Clupea pallasi*) lay its eggs in irregular masses largely on submerged aquatic marine vegetation. Irregularities in packing of the eggs in the mass produce discontinuities, or voids, among the eggs. A linking of voids into channels, if they occur, would provide a means by which natural convection could supply oxygen to eggs in the interior of the mass. Further, such convection probably would be more successful for egg masses on vertical surfaces such as the eelgrass normally used by spawning herring, compared with survival of eggs in masses laid on flat thallus-like surfaces of other forms of marine vegetation that are sometimes used.

Finally, in salmon egg respiration the vertical streaming of water over the egg surface, and the downward flow resulting from natural convection, raise certain questions regarding the efficiency of current designs of salmon egg incubators. Most salmon egg incubation boxes (Bams and Simpson 1977) and hatchery egg trays force water to upwell through the eggs or egg–gravel mixture. Under such conditions, the vectors associated with natural and forced convection are opposed and may tend to cancel each other. In a "downwelling" system, the two vectors may in part reinforce each other, suggesting a means of increasing the efficiency of oxygen transfer while using lower bulk transport velocities. If such lower bulk transport rates were proven not to compromise egg and alevin quality in artificial incubators, then the reduced water requirements would lead to lower power and filtration costs. Alternatively, alternating downwelling and upwelling modes of operation of gravel incubation boxes might also assist in maintaining higher permeability of the gravel through removal of accumulating fine particles. We believe these possibilities deserve further examination.

Acknowledgments

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Prediction of Egg Development Times of Freshwater Copepods

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COOLEY, J. M., AND C. K. MINNS. 1978. Prediction of egg development times of freshwater copepods. J. Fish. Res. Board Can. 35: 1322–1329.

Available literature data on the relationship between freshwater copepod egg development time and temperature were summarized and fitted where possible to Bělehrádek's temperature function

$$D = a(T - \alpha)^b$$

where D is development time, T is temperature, a, b, and α are fitted constants. In the calanoids, and to a lesser extent cyclopoids, a and α were predictably related as were α and in situ egg hatching temperature (T_H) when b was assumed to be constant for all copepods. This information was used to describe a simple procedure whereby a curve relating egg development and temperature could be constructed by only knowing T_H and applying it to Bělehrádek's temperature function.

Key words: Calanoidea, Cyclopoidea, zooplankton, temperature, production assessment

COOLEY, J. M., AND C. K. MINNS. 1978. Prediction of egg development times of freshwater copepods. J. Fish. Res. Board Can. 35: 1322–1329.

Les données publiées sur la relation entre la durée du développement d'oeufs de copépodes d'eau douce et la température ont été résumées et appliquées, quand la chose était possible, à la fonction de température de Bělehrádek,

$$D = a(T - \alpha)^b$$

où D est la durée de développement, T la température, a, b et α sont des constantes ajustées. Chez les calanoïdes et, à un dégré moindre, chez les cyclopoïdes, a et α montrent une relation prévisible. Il en est de même de α et de la température (T_H) d'éclosion des oeufs in situ quand on suppose que b est constant pour tous les copépodes. A partir de cette information, nous décrivons une méthode simple selon laquelle on peut construire une courbe reliant le développement de l'oeuf et la température quand T_H seulement est connu et en l'appliquant à la fonction de température de Bělehrádek.

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CONSIDERABLE attention has been given to the relationship between temperature and development time of the eggs and instars of freshwater zooplankton. Besides further elucidating the relationship between an organism and its environment, these data have often been used in production assessment techniques (Edmondson and Winberg 1971; Bottrell et al. 1976). Since establishing this relationship is a lengthy process, we analyzed published data to examine the possibility of predicting the relationship in a species for which experimental data were lacking.

Bělehrádek's equation $D = a(T - \alpha)^b$ (D = development time, T = temperature, a, b, and α as fitted constants) has been used for egg development rate data as a means of interpolating times at other than experimental temperatures. The equation's utility has been demonstrated by McLaren (1963, 1966), McLaren et al. (1969), and others. The three fitted constants in the equation can account for three ways in which monotonic responses to temperature may differ: a accounts for differences in elevation by defining shifts along the y or development axis; b depicts the degree of curvilinearity of the response; and α, sometimes referred to as "biological zero," is the theoretical temperature at which development time becomes infinite, and accounts for shifts of the response curve along the temperature or x axis. McLaren (1966) has shown that α is related to environmental temperature by assuming b is the same among different populations of closely related marine copepods. It is the primary purpose of this paper to show that a similar predictable relationship exists for freshwater calanoids and to a lesser extent for cyclopoids. In addition, a simple relationship is observed

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TABLE 1. A list of the species and data sources used in this paper. Species numbers were assigned arbitrarily and are repeated in Table 3. (*N_1 development data also available in same paper [see Table 3].)

Species no.	Species	Location	Author
		Calanoid eggs	
1.	*Eudiaptomus gracilis*	Bodensee–Obersee, Germany	Elster (1954)
2.	*E. gracilis*	Schluchsee, Germany	Eckstein (1964)
3.	*E. gracilis*	Bough Beech Reservoir, England	Munro (1974)
4.	*E. graciloides*	L. Erken, Sweden	Nauwerck (1963)
5	*E. graciloides*	L. Esrom, Denmark	Bosselmann (1975)
6.	**Acanthodiaptomus denticornis*	Titisee, W. Germany	Eichhorn (1957)
7.	**Mixodiaptomus laciniatus*	Titisee, W. Germany	Eichhorn (1957)
8.	*Arctodiaptomus salinus*	Nozha Hydrodome, Egypt	Elster et al. (1960)
9.	*Leptodiaptomus pallidus*	Strip Mine Ponds, Missouri, USA	Geiling and Campbell (1972)
10.	**L. minutus*	Bluff L., N.S., Canada	Cooley (1973)
11.	**Aglaodiap. spatulocrenatus*	Bluff L., N.S., Canada	Cooley (1973)
12.	*A. clavipes*	Farm Pond, Oklahoma, USA	Robertson et al. (1974)
13.	*Skistodiaptomus reighardi*	Black Pond, Ont., Canada	Carter (1974)
14.	**S. oregonensis*	Teapot Lake, Ont., Canada	Rigler and Cooley (1974)
15.	*Limnocalanus macrurus*	Char L., N.W.T., Canada	Roff (1972)
		Cyclopoid eggs	
16.	*Cyclops vicinus*	Bodensee, Germany	Spindler (1971)
17.	*C. vicinus*	Bough Beech Reservoir, England	Munro (1974)
18.	*C. abyssorum*	English Lake District, England	Smyly (1974)
19.	*C. abyssorum*	L. Trawsfyndd, England	Lewis (1971)
20.	*C. abyssorum*	Titisee, W. Germany	Szymanski-Bucarey (1974)
21.	*C. scutifer*	Latnajure, Sweden	Taube (1966)
22.	*Mesocyclops leukarti*	English Lake District, England	Smyly (1974)
23.	*M. leukarti*	L. Erken, Sweden	Taube (1966)
24.	*Acanthocyclops viridis*	Dublin Ponds, Ireland	Kahn (1965)
25.	*A. vernalis*	Aquarium culture, Oklahoma, USA	Robertson et al. (1974)
26.	*Eucyclops agilis*	Thames River, England	Bottrell (1975)
27.	*Thermocyclops hyalinus*	L. George, Uganda	Burgis (1970)
28.	*Mesocyclops edax*	Black Pond, Ont., Canada	Carter (1974)
29.	*M. edax*	Bay of Quinte, Ont., Canada	Cooley (1978)
30.	*Diacyclops bicus. thomasi*	Bay of Quinte, Ont., Canada	Cooley (1978)

that will allow estimation of an egg development curve in the absence of experimentation.

Materials and Methods

Bělehrádek's equation was fitted to published data on the relationship between temperature and development time of subitaneous eggs and first naupliar instars (N_1) of a number of limnetic copepods (Table 1). Data on development at those temperatures that authors cited as producing abnormalities such as lack of egg formation or greater than 50% embryonic mortality, were excluded.

Because the data were obtained from many different authors who employed different experimental methods our confidence in each data set is variable. For example, some authors obtained development times of individual egg sacs at many "fixed" temperatures which varied in some cases by as much as ±2°C (Elster 1954; Eichhorn 1957) while others restricted themselves to a few egg sacs at several temperatures (Carter 1974; Geiling and Campbell 1972). In several cases (Eckstein 1964; Elster et al. 1960) interpolated temperatures from smoothed curves were given instead of raw data. For some species a best estimate of b could not be obtained by fitting Bělehrádek's equation to the data.

Where possible, we fitted regressions to the raw data rather than using mean development times for all egg sacs at a given temperature. In some of these instances the number of points used to fit the regressions was quite high as for example in Rigler and Cooley's (1974) data on *Skistodiaptomus oregonensis* even though only five experimental temperatures were used. More often, however, only average egg development times are presented in papers, which meant that Bělehrádek's equation was fitted to only a few points (see columns 7 and 9, Table 3).

In several instances curves describing egg development were drawn showing average development times at several temperatures, but not citing actual values (Nauwerck 1963; Spindler 1971; Lewis 1971; and Bosselmann 1975). We photographically enlarged these figures to allow more exact interpolation of data points.

Some data sets required subjective interpretation before Bělehrádek's equation could be fitted. These are described briefly below.

Extensive data on *Aglaodiaptomus clavipes* and *Cyclops vernalis* are given by Robertson et al. (1974). Although a summary is given for "mean clutch carrying times" at four temperatures for *A. clavipes* in their table 21, we preferred to fit the function to the raw data listed in their appendices B1 to B4 inclusive. The authors cite lengths of time in-

TABLE 2. Egg development times for *Leptodiaptomus minutus* fitted to Bělehrádek's equation in several different ways.

1	2	3	4	5	6	7	8
				Predicted egg development (h)			
Temp. (°C)	No. of egg sacs	Obs. mean dev. time (h)	SD	With $n = 6$ Best fit $b = -2.00$		With $n = 63$ Best fit $b = -2.00$	
4	20	490.	15.1	496.	494.	491.	493.
7	16	302.	10.2	296.	294.	297.	297.
10	4	191.	1.8	194.	194.	196.	198.
14	5	122.	2.2	122.	125.	123.	128.
18	9	82.	1.1	83.	87.	83.	90.
23	9	56.	1.5	55.	60.	54.	62.
				$a = 159632.$	50430.	355133.	53330.
				$b = -2.32$	-2.00	-2.52	-2.00
				$\alpha = -8.05$	-6.1	-9.64	-6.4

dividual females, hatched at varying temperatures, carry successive egg sacs at each of four egg incubation temperatures. We have averaged carrying times for each female and fitted these values at the appropriate incubation temperature to Bělehrádek's function. For *C. vernalis*, we fitted all the data in their table 56 which listed estimates of egg development time at five temperatures, and which included 3 egg sac development times ±1 day at 5°C to 33 egg sacs each at 26 and 31°C ± approximately 8 h.

In Taube's (1966) thesis there are inconclusive egg development data on *Cyclops scutifer* suggesting times of 625 and 380 h at 5 and 8°C, respectively. A somewhat subjective interpretation of the data at 2°C by the author suggests development time to be 1500 h. She suggests this estimate "seems reasonable when compared to the values for the higher temperatures."

Several copepod egg development curves have been omitted from the summary in Table 1 and from our analyses. Cummins et al. (1969) present semi-log regressions of egg development times on temperature for *Leptodiaptomus siciloides* and *Cyclops vernalis* determined from field data on peaks and lows in mean clutch sizes and average lake water temperatures. Einsle (1968) presents a smoothed egg development curve for *Mesocyclops leukarti* but no raw data or interpolated values are given, and we are reluctant to interpret development curves for iterative analyses unless experimental data points at specific temperatures are available. Gras (1970) presented egg development data on *Tropocyclops incognitus* at only two temperatures.

The "best fit" of Bělahrádek's equation was determined using the least squares method with the logarithmic form of the equation:

$$\ln D = \ln a + b \ln (T - \alpha).$$

Values of α were iterated to minimise the residual sum of squares.

For the "fixed-best fit" of the equation, a regression through the origin was used (Steele and Torrie 1960, p. 179). Once the value of b in the equation has been specified, Bělehrádek's equation is reduced to a linear form where D is a scalar multiple of $(T - \alpha)^{-2.0}$. The use of an ordinary regression on the linear or logarithmic form would be inappropriate because only one parameter is to be estimated rather than two. As before α was iterated to minimise the residual sum of squares.

Results

As with any multiparameter equation, in obtaining the best fit to Bělehrádek's equation, small changes in the data can cause large changes in the fitted constants. This low resolution is illustrated in Table 2 with data on egg development for *L. minutus*. If mean values at six experimental temperatures in column 3 are fitted to Bělehrádek's equation, the resulting b, α, and especially a values in column 5 are very much different than if the 63 individual egg development times are fitted to the same equation, as seen in column 7; but in both cases the resulting equations from these "best fits" are empirically adequate in describing the data. If both these data sets are again forced to fit Bělehrádek's equation but with b held at a much lower value of -2.0, the resulting fits in columns 6 and 8 still adequately describe the observed data even though the values of a are much reduced to around 50 000. McLaren (1963) has observed that the resolution of Bělehrádek's function can be increased by assuming that b is constant for related groups. The resulting regularization of the constant of proportionality that occurs from this procedure reduces the spread of iterated a values from 14 000 to 68 000 instead of 2000 to values approaching infinity (Table 3). This does in fact seem like a more logical spread of values in a closely related group such as freshwater limnetic copepods. This "justifies" the procedure from an intuitive point of view rather than a statistical one as has been demanded by critics (Heip 1974; Bottrell 1975). A procedure that produces constants that are useful from an empirical viewpoint need not be justified theoretically. Thus if the iterated α's are a reflection of temperature adaptation but are not a true "biological zero" the usefulness still remains.

For the calanoids and cyclopoids listed, the average b values are -1.97 and -1.40, respectively, or -1.72 for all copepods. Because of the variability in methodologies, we chose not to weight best estimates of b in arriving at a common b for a particular group. However, within wide limitations the choice of an average

TABLE 3. A summary of the data compiled and used in developing a procedure to estimate egg development times.

1	2	3	4	5	6	7	8	9	10
Spec. no.	Best fit of Bělehr. function			Fixed b		No. of temp.	Experimental temp. range	No. of pts.[c]	Hatch temp. (T_H)
	a	α	b	a	α				
				Calanoid eggs					
				$b = -2.0$					
1.	14289.	−2.76	−1.73	39080.	−4.8	C[a]	5–23	59	4
2.	21193.	−4.85	−1.82	41532.	−6.1	I[b]	2–29	16	2
3.	11060947.	−13.59	−3.50	37953.	−4.8	4	5–20	4	4
4.	20193.	−2.52	−1.86	34162.	−3.4	4	6–22	4	13
5.	2473633.	−12.70	−3.07	50307.	−6.8	I	2–20	97	—
6.	48064.	−3.13	−2.06	38130.	−2.7	C	7–22	15	13
7.	6892.	−2.77	−1.46	43603.	−6.0	C	3–23	59	4
8.	453961.	−10.78	−2.53	54045.	−6.0	I	11–29	20	15
9.	351.	+7.77	−0.77	19970.	−0.3	4	10–25	4	—
10.	349637.	−9.61	−2.51	53330.	−6.4	6	4–23	63	2
11.	314446.	−5.73	−2.59	44199.	−3.2	6	4–23	37	11
12.	376.	+9.91	−0.85	25438.	−1.0	4	14–31	28	—
13.	2311.	+4.08	−1.34	21406.	0.0	6	10–31	5	15
14.	53223.	−4.48	−2.11	35489.	−3.8	5	4–23	173	9
15.	917.	−3.35	−0.31	992078.	−40.3	I	2–10	4	—
				Cyclopoid eggs					
				$b = -1.4$					
16.	1163.	+3.26	−1.04	4098.	−0.1	6	9–26	6	—
17.	7.416×10^{13}	−46.13	−6.70	4764.	−2.8	4	5–20	4	4
18.	1.499×10^{11}	−82.10	−11.44	6766.	−3.3	4	4–16	4	4
19.	2.09×10^{12}	−94.09	−12.52	7636.	−3.2	3	5–15	3	6
20.	17291.	−4.43	−1.63	7774.	−2.8	10	4–23	147	3
21.	4936.	−0.74	−1.18	8665.	−1.5	3	2–8	3	4
22.		can't fit		4335.	+0.4	3	8–16	3	9
23.	12102.	−0.43	−1.77	3559.	+1.8	4	8–25	4	10
24.		can't fit		2563.	+2.0	5	10–30	5	—
25.	3622.	−0.31	−1.45	3040.	+0.0	4	5–26	52	—
26.	16130.	−3.88	−1.67	6135.	−1.8	4	5–20	4	—
27.	823.	+9.51	−1.12	2109.	+7.3	4	15–30	7	25
28.	556.	+8.07	−0.91	2136.	+6.0	5	10–21	5	16
29.	6736.	+1.69	−1.50	4809.	+2.3	5	7–27	5	—
30.	18932.	−4.79	−1.77	4755.	−1.7	7	4–27	7	2
				Calanoid N_1					
				$b = -2.0$					
6.	2523.	−1.08	−1.62	27599.	−3.6	C	3–22	36	
7.	2313.	−3.60	−1.65	35072.	−7.6	C	2–22	18	N/A
10.	23792.	−7.08	−2.19	66131.	−8.9	6	4–23	77	
11.	204552.	−9.39	−2.83	59671.	−8.1	5	4–18	27	N/A
13.	2300251.	−14.63	−3.34	61819.	−8.0	5	4–23	101	

[a]C = estimates of egg development time made at a continuum of temperatures over the experimental temperature range.
[b]I = egg development times interpolated by the original author.
[c]The number of points used to fit the regression.

or "true" b for a related group of species is not critical for subsequent iteration procedures of a. For this reason we chose, as a convenience only, to use $b = -2.0$ for the calanoids. In fact the data for the calanoid species are in general much better because more egg sacs were tested at more temperatures for more species in the original experiments.

In order to test McLaren's suggestion that α is related to environmental temperature in closely related forms (when b is held constant) α has been plotted against the water temperature when the first generation of subitaneous eggs hatch and survive (Fig. 1). It is important to realize that many of these species may have first naupliar stages developing in water cooler than indicated in column 10 of Table 3, but these animals will have hatched from resting eggs produced at

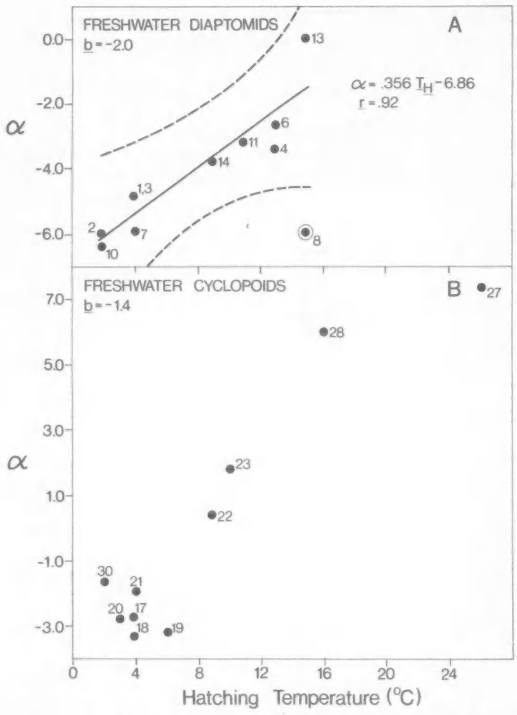

FIG. 1. Alpha from Bělehrádek's equation plotted against hatching temperature. A, Freshwater diaptomids. B, Freshwater cyclopoids. The numbers refer to species listed in Tables 1 and 3. The data on *Arctodiaptomus salinus* (#8) in A were not used in calculating the regression. 95% confidence limits are presented as dashed lines.

an earlier time and deposited on the sediments. These hatching temperatures are not available for all species in Table 3. We have restricted ourselves to data that include water temperatures, and population dynamics for a species' egg development curve from the same lake. In general lower α values are associated with species whose subitaneous eggs are likely to develop in colder water at some time in their life cycle. In Fig. 1A *Arctodiaptomus salinus* (#8) is conspicuous by its deviation from the line and there are several possible reasons for this. The data used to fit the curve were interpolated from a curve drawn by Elster et al. (1960) that, at best, seems to be highly subjective since there is a lot of scatter at the four experimental temperatures. Second, these data are from a lake that is brackish while all other lakes in Table 1 are fresh. When α for *A. salinus* is compared with marine copepod species and an "average environmental temperature" (McLaren et al. (1969), their average b of -2.05 was nearly the same as ours, see their fig. 3), the fit is better. Therefore, it is possible that in this lake the temperature adaptation of the egg development curve is more aligned with marine than limnetic copepods.

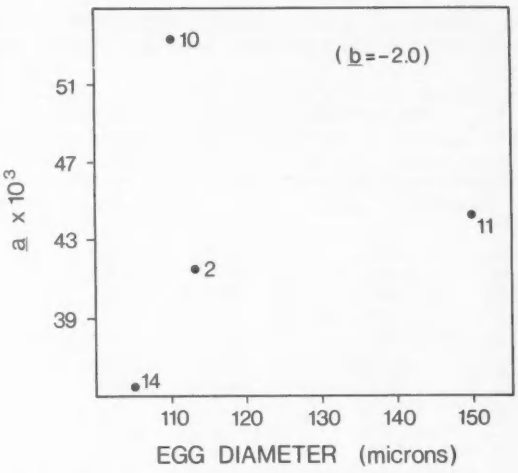

FIG. 2. A plot of a from Bělehrádek's equation and egg diameter for freshwater diaptomids. The numbers refer to species listed in Tables 1 and 3.

Limnocalanus macrurus could not be placed on Fig. 1A with the rest of the calanoid group since the iterated α was greater than the lowest experimental temperature (i.e. 0°C) when b was fixed at -2.0. This is not surprising in view of its designation as a glacial relict. Its α would probably best be compared with other development curves constructed from eggs collected over its temperature range and perhaps other glacial relicts such as *Mysis relicta* and *Senecella calanoides*.

If it is true for freshwater diaptomids that α can more or less be estimated from lake water temperature and b can be assumed to be $= -2.0$ then a need only be known in order to produce a formula to predict egg development. McLaren (1966) found a was related to egg diameter in some marine copepods but these data were not generally available in our literature search. The four egg diameters we found suggested there was no predictable or empirically useful relationship (Fig. 2).

Since α and a are interrelated we thought they might be related in a useful empirical fashion. The plot of a versus α (Fig. 3) once again shows that a significant linear relationship exists for the calanoids, a weaker one for the cyclopoids.

Encouraged by these results we tried the same type of plot on other groups of related species for which Bělehrádek's type analyses were available. Several extreme results are illustrated for two dissimilar groups; varieties of the frog *Rana pipiens* from North and Central America (McLaren 1965) and decapods from England (Wear 1974) (Fig. 4A and B). Two striking relationships are evident in the frog data where each plotted point represents a "thermal race." There is one relationship between a and α for continental U.S. forms primarily from the east coast region, and another for

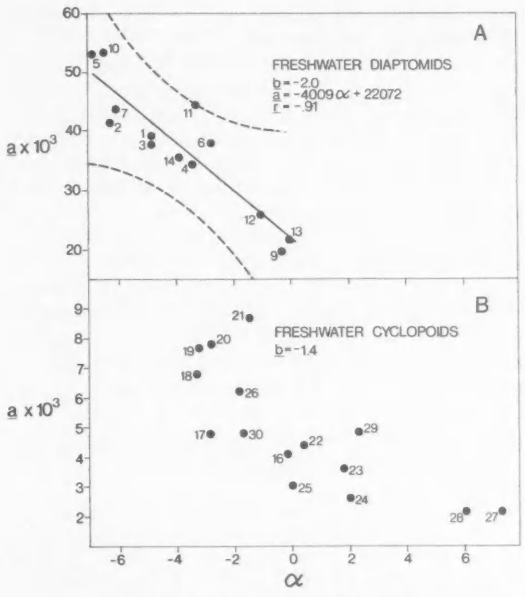

FIG. 3. A plot of a versus α from Bělehrádek's equation. A, Freshwater diaptomids. B, Freshwater cyclopoids. The numbers refer to the species listed in Tables 1 and 3. 95% confidence limits are presented as dashed lines.

Central American forms. Reassuringly, races from the Southern states of Texas and Florida fall near the middle. There is no such obvious relationship between a and α for the decapods.

Discussion

We were prompted to analyse these literature data on egg development in copepods for several reasons. These data are widely used to estimate recruitment and ultimately production in several different methods (Edmondson and Winberg 1971) but they are difficult to obtain for a particular species because of the long incubation times required under exacting experimental conditions. In many instances it may be acceptable to estimate an egg development curve for a calanoid species using the simple relationship we have described. If, for some reason, very precise information relating development time and temperature is required, then the data should be collected for the species in question.

Apparently a given species' egg development curve can change depending on the lake. This is clearly illustrated in the two different curves produced from populations of *Eudiaptomus gracilis* in the Bodensee–Obersee and the Schulchsee (Elster 1954; Eckstein 1964). At 4°C the Schluchsee eggs take approximately 410 h to develop while the Bodensee–Obersee eggs require almost 525 h. These differences in development rate gradually disappear as incubation temperature increases. At 10°C the times are 161 and 175 h, and at

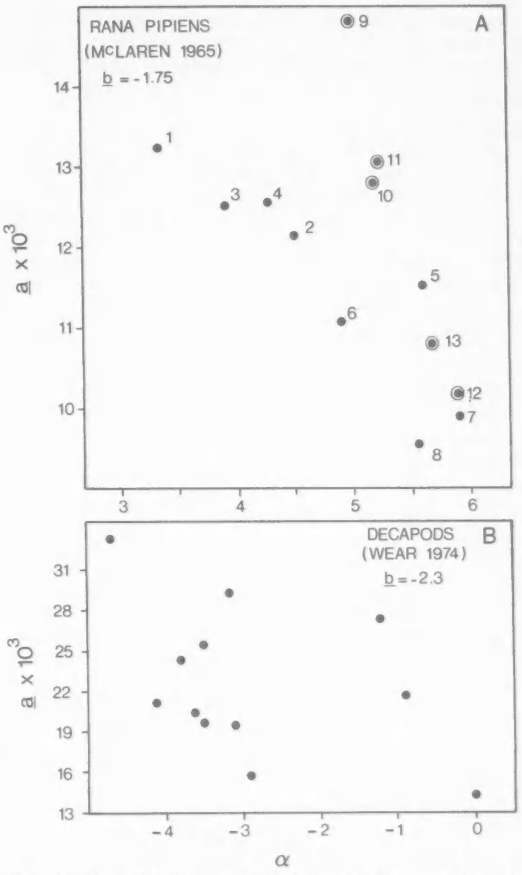

FIG. 4. A polt of a and α Bělehrádek's equation. A, McLaren's data on the frog, *Rana pipiens*, assuming $b = -1.75$. The numbers refer to the species he lists sequentially in his Table 1. The numbers in circles are species from Mexico and Central America. B, Wear's data on British decapods assuming $b = -2.3$.

20°C the times are almost identical, 63 and 64 h, respectively.

In the calanoids, in absolute terms the greatest differences in egg development times occur at colder temperatures but there is a great deal of variation in development rate, even at midrange temperatures. Of the 13 freshwater diaptomids listed in Table 1, the estimates fall between 62 and 89 h at 18°C and between 161 and 248 h at 10°C. Development time estimates using the constants in columns 2, 3, and 4 of Table 3 are not comparable for all diaptomids at colder water temperatures since in many cases the experiments did not involve lower incubation temperatures (see column 8). The variation in estimates for those species that were successfully incubated at 5°C is striking. *Eudiaptomus graciloides* from Lake Esrom in Denmark apparently takes only 364 h to develop whereas the eggs from the

"summer" diaptomid *Aglaodiaptomus spatulocrenatus* in Bluff Lake, N.S., take 675 h. It should be noted, however, that gravid females of this latter species apparently never experience these water temperatures in Bluff Lake.

The data are not as complete for the cyclopoids but the variation is apparently of the same magnitude. The eggs of *D. b. thomasi* require only 333 h at 5°C while those of *C. scutifer* take 670 h. At 18°C there is almost a 2 fold difference in development time between the eggs of *A. vernalis* which requires 55 h and *C. abysorum* requiring 105 h from the Titisee in West Germany.

The limited data on calanoid N_1 development also indicate there is a great deal of variability in development time in this stage. First naupliar instars of *Mixodiaptomus laciniatus* take only 69 h to moult at 5°C while those of the "spring and summer" species *S. oregonensis* require 111 h. All five calanoid species in Table 1 require between 15 and 20 h to complete N_1 development at 18°C.

These data make it clear that no one universal egg development curve can be applied to either calanoids or cyclopoids and that there are significant variations even within a species.

For the freshwater diaptomids at least, the empirically demonstrated relationships between egg hatching temperature (T_H) and α and a and α when b is constant seem to be more than fortuitous. It should be possible then to exploit these relationships in a simple procedure which would allow one to derive a Bělehrádek type equation relating egg development and temperature in the following manner:

1. assume $b = -2.0$

2. estimate coldest water temperature (T_H) when subitaneous eggs hatch (and survive) from population dynamics and lake isotherm data

3 using T_H and relationship in Fig. 1, estimate a species' α from

$$\alpha = .356T_H - 6.86 \qquad \text{Equation 1}$$

4. using the estimated species α, estimate a from the relationship pictured in Fig. 3 as described by

$$a = -4009\alpha + 22072 \qquad \text{Equation 2}$$

5. having assumed b and estimated α and a Bělehrádek's equation can be assembled.

Our procedure was developed as a result of the variability observed in egg development curves in even the same species but will yield a relationship that at best is an approximation of the real thing. The estimation of the water temperature when the first clutch of subitaneous eggs hatch is obviously important in predicting curves, but we do not see that this first step need be done rigorously.

The data we have analysed indicate that eggs of the same species in different environments will likely have different responses to temperature. This reflects a local acclimatization to the environment but we do not know of data which would indicate the time scale over which this temperature response occurs.

The relationships between a and α pictured in Fig. 3 and 4 are curious. The limited data available suggest this relationship occurs only in closely related groups such as races of a particular species or different species of a particular genus. In large diverse groups (e.g. Decapoda) the relationship is very ill defined; at least it is useless in an empirical sense.

We feel that this simple procedure which is dependent only on estimating T_H for a species will generate an egg development curve that will in many cases be adequate for estimating egg turnover. Our data suggest that curves derived in this manner could be more indicative of the true relationship than by assuming a universal egg response to temperature (Bottrell et al. 1976).

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Generation Lengths of Some Temperate Marine Copepods: Estimation, Prediction, and Implications

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McLaren, I. A. 1978. Generation lengths of some temperate marine copepods: estimation, prediction, and implications. J. Fish. Res. Board Can. 35: 1330–1342.

Only copepodids should be used to trace synchronous cohorts from relative abundance of stages, and only long-lived adults correctly signal new generations from size changes. From extensive published data from Loch Striven, Scotland, life cycles are thus detailed for *Pseudocalanus minutus*, *Microcalanus pygmaeus*, *Calanus finmarchicus*, *Centropages hamatus*, *Temora longicornis*, *Acartia clausi*, and *Oithona similis*. Generation lengths are also estimated for all but *M. pygmaeus* from temperatures in nature and from laboratory data on food-satiated development. For stage durations (D) at various temperatures (T), Bělehrádek's temperature function $D = a(T - \alpha)^{-b}$ is used. Temperature response can be about the same throughout a species range. With b fixed, α within a species can be the same for older stages as for embryonic duration, which can thus be used to estimate a for generation length even from a laboratory example at a single temperature. If food-satiated durations are available only for some stages, it can be assumed that other stages are similar (isochronal development). Food-satiated generation lengths predicted thus from the laboratory match those inferred from the Loch Striven samples. Trophic studies may be less revealing than further work on the "intrinsic" determinants of copepod performance. Competition for food should not be assumed in studies of niches and community dynamics of marine copepods.

Key words: Copepoda, generation lengths, cohorts, body sizes, prediction, temperature, production, life cycles

McLaren, I. A. 1978. Generation lengths of some temperate marine copepods: estimation, prediction, and implications. J. Fish. Res. Board Can. 35: 1330–1342.

On ne devrait utiliser que des copépodides pour déceler des cohortes synchrones à partir de l'abondance relative des stades, et seuls les adultes à longue durée de vie annoncent correctement de nouvelles générations à partir des changements de taille. Nous décrivons donc en détail, utilisant les nombreuses données publiées du Loch Striven, Ecosse, les cycles biologiques de *Pseudocalanus minutus*, *Microcalanus pygmaeus*, *Calanus finmarchicus*, *Centropages hamatus*, *Temora longicornis*, *Acartia clausi* et *Oithona similis*. Nous estimons également la durée des générations pour toutes les espèces, sauf *M. pygmaeus*, à partir des températures dans la nature et de données de laboratoire sur le développement avec nourriture à satiété. Nous utilisons la fonction de température de Bělehrádek's, $D = a(T - \alpha)^{-b}$, pour déterminer la durée des stades (D) à diverses températures (T). La réaction à la température peut être à peu près la même chez toute une gamme d'espèces. Si b a une valeur fixe, α au sein d'une espèce peut être semblable aux stades âgés et aux stades embryonaires. On peut donc utiliser cette dernière valeur dans l'estimation de a pour la durée de génération, même à partir d'un exemple de laboratoire à une seule température. Si les durées avec nourriture à satiété ne sont disponibles que pour quelques stades, on peut supposer que les autres stades sont semblables (développement isochrone). Les durées de génération avec nourriture à satiété prédites de cette manière en laboratoire s'accordent bien avec celles qui ont été déduites à partir des échantillons du Loch Striven. Il se peut que des études trophiques soient moins révélatrices que des travaux plus poussés sur les déterminants « intrinsèques » de la performance des copépodes. On ne devrait pas supposer de concurrence pour la nourriture dans des études de niches et de dynamique des communautés de copépodes marins.

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This paper is based entirely on published data and analyses, but is not simply a review. Rather, the aims are 1) to outline more efficient ways of analyzing synchronous life cycles of copepods, 2) to develop some techniques for estimation of demographic and production parameters from limited laboratory data, 3) to show from these techniques that generation lengths of some widespread temperate, marine species may be independent of food supply for long periods, and 4) to consider implications of such a conclusion for some current approaches in plankton research.

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People have been counting and measuring copepods for a long time, generally hoping to elucidate life cycles from cohorts (broods or generations, sensu McLaren 1969) in successive samples. A life cycle can be described as an end in itself, or production can be measured by the "cohort method" (Winberg 1971), especially for slowly, synchronously developing copepods at high latitudes (e.g. McLaren 1969) or in lakes in general (e.g. Rigler and Cooley 1974). However, only a few current workers (e.g. Gaudy 1976; Razouls 1974) seem to believe that synchrony of cohorts justifies the laborious counting and measuring of marine copepods at lower latitudes, especially in the open sea. However, metabolic and trophic studies have flourished. An original strategy of such research, sometimes lost sight of, was to develop techniques for estimating secondary production in nature.

Soviet investigators in estimating secondary production in the open sea in many parts of the world routinely make bold approximations from laboratory development rates, often based on limited rearings of copepods (reviews in Winberg 1971). Their precise techniques are not always made explicit and the parameters that they use in production/biomass ratios are often buried in earlier works, difficult to obtain. More seriously, they have not generally shown that the laboratory rates, supposed to be food-satiated, actually apply in nature.

In a recent review of the biology of *Pseudocalanus*, Corkett and McLaren (1978) used the fine study by Marshall (1949) of samples of copepods taken weekly between midwinter and early autumn, 1933, from Loch Striven, Scotland. The frequency of sampling together with the large amount of tabulated data are unique in the marine copepod literature. By analyzing Marshall's counts and measurements, Corkett and McLaren were able to show that the life cycles of *P. minutus* in this isolated, fiord-like setting were even more synchronous than she had supposed, and that its generation lengths were similar to those predicted from food-satiated animals in the laboratory. They also suggested that the same independence of food supply occurred in other temperate localities.

Here the extensive data on other species of copepods in Loch Striven are examined to see if their life cycles can similarly be defined and related to predictions from the laboratory. All the species studied by Marshall (1949) and Marshall et al. (1934) are included, with the exception of *Paracalanus parvus*, which was present only for a brief period, too short for useful analysis.

Materials and Methods

Detecting Generations in Nature

From cohorts — The methods used here are not new, but are refinements of widely used approaches. Although absolute counts of different stages can be used to trace cohorts in samples from small, enclosed bodies of water (e.g. Carter 1965; McLaren 1969; Rigler and Cooley, 1974), sampling variations and patchiness are of importance in marine studies. For this reason, detailed counts of copepod stages are often graphed as relative abundances (e.g. Marshall 1949; Gaudy 1976; Razouls 1974). However, three logical constraints are often unheeded. 1) The inclusion of stages with widely different durations in the plankton can mislead. For example, adults may be long-lived and younger stages ephemeral, so that the accumulated and sustained abundance of adults may overwhelm changes in relative abundance of younger stages. 2) The inclusion of stages subject to different sampling efficiencies can also mislead. For example, coarse nets when clogged might filter nauplii more efficiently, whereas fine nets when clogged might allow more of the older, more mobile stages to escape; both circumstances might give an unreal increase in nauplii, to be interpreted as a recent "spawning." 3) The inclusion together of stages that have widely different mortality patterns could give false cohorts. For example, excessive deaths of nauplii could result in a large increase in relative abundance of copepodids.

Considering the above constraints, it can be argued that only the copepodid stages are more-or-less comparable among themselves for seeking cohorts from plots of relative abundances. Marshall (1949) and Marshall et al. (1934) plotted the relative abundance of each stage (except eggs) as a percentage of the total and, as will be shown, were only partly successful in recognizing cohorts. In fact, only one copepodid stage need actually be identified among those counted. If the population is reasonably synchronous, the relative abundance of the chosen stage should show well-defined pulses through time. Rigler and Cooley (1974) show how the time of appearance of a cohort in a stage should be given by the mean time from integration of numbers through time. This would be particularly important when development is not synchronous, i.e. when there are "stragglers." However, in the present paper time of appearance of a cohort in copepodid stage V (C V, except for *Pseudocalanus minutus*, in which C IV is used) is simply defined as time of the peak in relative abundance. This is not as accurate as the method of Rigler and Cooley, but has the advantage for present purposes of being easily confirmed by the reader.

From body sizes — Generations can also be distinguished entirely independently of relative abundance of stages by use of variations in body sizes. Cephalothorax lengths of copepods vary seasonally, probably largely or entirely due to changes in temperature (McLaren 1963, 1974; Miller et al. 1977). Lengths of copepodids as well as adults have been measured in many seasonal studies. However, much of this effort may not reveal generations: if copepodid stages develop continuously throughout a period when temperatures also change continuously, then changes in size of these stages should also be continuous, the discontinuities merely imposed by sampling intervals. However, adult copepods do not molt after maturing and adult females, at least, can be quite long-lived. The following sequence should be found where life cycles tend to be synchronous and temperatures are changing: a period of stability in size of adult females should be followed by rapid change, which then should be followed by a period of stability at a new size after the old generation is numerically overwhelmed by the new. Adult females should generally change size after the peak in the copepodid cohort (see above). However, obvious size changes may be delayed if the new generation

is relatively scarce. Conversely, obvious size changes may occur early if a new generation is relatively abundant or if synchrony is not very marked. Examples will be given to support these predictions.

Digby (1950) was among the first to interpret size-frequency histograms of female copepods in following successive generations. Recently, Evans (1977) expressed dissatisfaction with such qualitative interpretations of histograms. He used, instead, an a posteriori statistical test to detect differences in female size and infer generations from samples of copepods from the North Sea. However, the high variances and nonnormal size-frequency distributions that often occur in copepod samples may obscure real changes between generations. In the present paper, appearances of new generations are inferred from shifts in hitherto stable or nearly stable modal size-classes on size-frequency histograms. The question of visual vs. statistical interpretation will be considered further, using *P. minutus* as an example.

Predicting Generation Lengths from Laboratory Data

The first problem concerns the generality of predictions from laboratory data. At worst, it could be that every local population manifests its own intrinsic rate processes. However, some evidence indicates that development rates, and hence generation lengths, of food-satiated copepods are often quite uniform within the same species. By "species" is meant morphologically similar forms, recognizing that in some ways geographically separate populations may differ physiologically (e.g. in response of size to temperature, see McLaren 1965) or be reproductively incompatible (e.g. Carrillo B.-G. et al. 1974). As an example of the uniformity within species, McLaren (1966) showed that widely separated populations of the copepods *Pseudocalanus* sp. and *Calanus finmarchicus* had similar embryonic durations at the same temperatures, and Corkett and McLaren (1978) confirmed that this is also true for older developmental stages of *Pseudocalanus*. In this paper, it is shown for some species and assumed for others that rates determined for populations elsewhere can be applied to the copepods of Loch Striven.

A second problem concerns choice of a temperature function for interpolating and extrapolating from limited data within a species. McLaren (1966) and McLaren et al. (1969) showed that embryonic duration (D) of a variety of copepods was well described by Bělehrádek's temperature (T) function,

$$D = a(T - \alpha)^{-b},$$

where a and α were fitted and b was held constant for all species (taking b as the weighted mean for all species). The conversion from a 3-parameter to a 2-parameter equation inevitably reduces the goodness of fit, but the purpose was empirical; a among different populations of the same species or among closely related species becomes almost proportional to egg diameter (McLaren 1966; Corkett 1972), and α becomes correlated with the mean environmental temperature to which the species is subjected (McLaren et al. 1969). In the present study, extensive use is made of another empirical convenience of the 2-parameter version of this equation. Corkett and McLaren (1970) showed that with b fixed at 2.05, the value of α determined for embryonic duration could be used in three species to describe the period from hatching to C I; that is, only a needed to be estimated for this period. This means (irrespective of the temperature function used) that the period from hatching to C I within species was a similar or the same multiple of embryonic duration at all temperatures. Landry (1975) and Corkett and McLaren (1978) showed in detail that this rule applied to a series of stages of *Acartia tonsa* and *Pseudocalanus* sp., respectively.

In this paper some theoretical generation lengths are accordingly derived for animals in nature from embryonic durations of the same species. By generation length is meant, not that of formal demography, but the time between appearances of the same stage of development in successive generations. From laboratory rearings, it is estimated here as the time between appearance of an egg and its maturation as an adult (female). This is valid if females produce eggs immediately after maturation; some species may (*Eurytemora herdmani*; McLaren 1976a) and others may not (*Pseudocalanus* sp.; Corkett and McLaren 1978). Arguing that size changes of females in nature might represent the "vanguard," rather than the norm, Corkett and McLaren (1978) used the period from hatching to maturity; here cohort peaks are interpreted in addition to size changes and the above, slightly longer period is used. It is even possible to predict generation lengths at all temperatures from a laboratory estimate at a single temperature. To do this, the expression $(T - \alpha)^{-b}$, with b and α estimated for embryonic duration, is divided into the single laboratory-estimated generation length at the temperature T, giving a value of a for generation length, and allowing prediction for other temperatures.

Another possibility for prediction of generation lengths from even more limited data has recently been supplied by Miller et al. (1977). They show that in well-fed individuals of two species of *Acartia*, all stages had about the same duration at given temperatures. They call this rule isochronal development. Although they suggest it may not apply to *Pseudocalanus*, Corkett and McLaren (1978) show that it does, quite well. In predicting generation lengths in this paper, it is assumed that isochronal development occurs in some species where it has not been demonstrated.

There remains the problem of estimating temperatures that influence generation lengths in Loch Striven. Marshall et al. (1934) list temperatures at 0 and 30 m; for purposes of estimation, and assuming that stratification was stronger near the surface, temperatures at 10 m were taken as intermediate between those at 0 and 30 m (Fig. 1). Marshall (1949) and Marshall et al. (1934) tabulate data from plankton hauls taken from the bottom (60 m) to 10 m and from 10 m to the surface. From these split hauls, plots for each species are given (upper panels, Fig. 2–8) of the proportions of nauplii and copepodids, respectively, that were taken above 10 m. This allows an estimation (repeatable by the reader) of mean temperature experienced during development of a generation as follows. When animals were virtually all above 10 m, they were assumed to have lived at temperatures intermediate between those shown for 0 and 10 m (Fig. 1). When they were about 50% above 10 m, temperatures at 10 m were used. When they were all below 10 m, temperatures at 30 m were used. When there were intermediate percentages above or below 10 m, interpolated temperatures were used. It was assumed that animals were nauplii during the first half and copepodids during the second half of a given generation period. The derived estimates of mean temperatures during each generation were then rounded to the nearest 0.5°C. The approach is a refinement of that chosen by Corkett and McLaren (1978), who simply

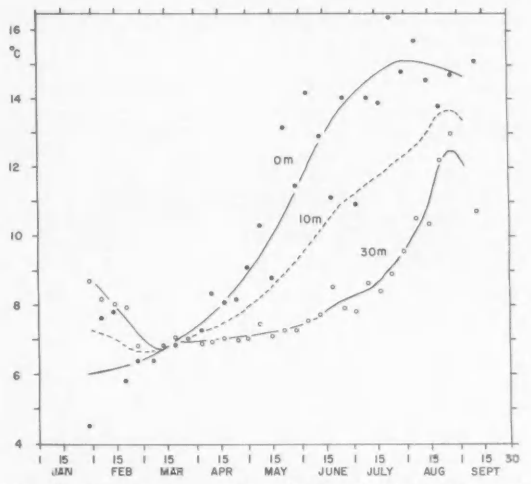

FIG. 1. Temperatures at 0 and 30 m in Loch Striven in 1933. Dashed line (mean of eye-fitted lines for 0 and 30 m) gives assumed temperatures at 10 m. Data from Marshall et al. (1934).

assumed that *P. minutus* lived closer to the surface than to 30 m, and used temperatures at 0 m. The present analysis attempts, however crudely, to acknowledge differences in depth distributions seasonally and among species.

Life Cycles of Copepods in Loch Striven

Pseudocalanus minutus

Marshall's (1949) account suggests that after the overwintered "brood" (generation) matured, five successive generations appeared: in early and possibly late April, late May, late June, and late August. Her conclusions about the earlier part of the season are broadly confirmed, but later events are substantially clarified (Fig. 2). A series of distinct peaks in the C IV cohort (ambiguous only for G_3) appeared at regular intervals through the season. By contrast, such peaks are obscured in the plots of Marshall (1949) of abundances of all stages (except eggs) as absolute numbers and as percentages of the total.

To assure readers that no material was selected for display, all size data given by Marshall (1949) are plotted on Fig. 2; for clarity in subsequent species (Fig. 3–8), some samples are excluded during periods when sizes were stable. In *P. minutus* every peak in a C IV cohort was accompanied by or followed shortly by a change in the dominant size mode of females. Subsequent to its first appearance in most generations, the dominant size mode remained consistent. The increase of size of females within G_0 was, as Marshall (1949) recognized, due to the maturation in the colder waters of winter of copepodids from the autumn. The deviations of size in G_1 were minor, and the pattern became confused after G_6. Between generations there

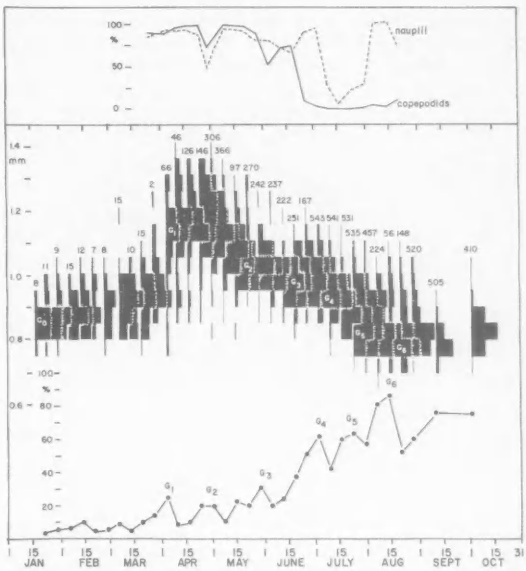

FIG. 2. Life cycles of *Pseudocalanus minutus* in Loch Striven; relative abundance of C IV as a percentage of all copepodids (*lower panel*), size and numbers per haul (including combined hauls from 60 to 10 m and 10 to 0 m) of adult females (*middle panel*), and percentage of nauplii and copepodids above 10 m (from split hauls, taken from 60 to 10 m and 10 to 0 m, *upper panel*). Successive generations as inferred from peaks in the C IV cohorts and size changes designated as G_0, G_1, etc. Data from Marshall (1949, tables III and X).

were distinctly transitional size-frequency distributions. The first generation (G_1) greatly outnumbered G_0 and size change of adults coincided with the peak in the C IV cohort. Subsequently, there was a delay of size change after passage of the C IV cohort, until later in the season. This may be, as suggested under *Materials and Methods*, because of reduced synchrony of cohorts as the season progressed. By contrast, Marshall's (1949) plot of mean sizes during the season lacks both sharp changes and periods of relative stability.

For a more formal analysis a probability matrix was calculated of differences among means of all female sizes tabulated by Marshall (1949). It revealed no differences at $P < 0.05$ between dates within G_1 through G_5 (as designated a priori on Fig. 2), but sharp breaks at $P < 0.01$ are found between these presumed generations. Significant differences were found within G_0 and significant discrimination between samples of G_5 and G_6 was evidently confounded by shifts in the nonnormal size-frequency distributions, in spite of stability of the modes. For these reasons it is felt that a visual display (Fig. 2) is more revealing than statistical analysis, which is not attempted for species subsequently dealt with in this paper.

TABLE 1. Estimated generation lengths of copepod species in Loch Striven, Scotland (based on data from Fig. 2, 4, 6, 7, and 8) compared with lengths predicted from temperatures during development (see text).

Species	Generation number	Dates of appearance of		Estimated temp (°C)	Generation length (d)	
		Peaks of C V cohorts[a]	Female size changes		From samples	Predicted
P. minutus	2	Apr. 4–May 1	Apr. 4–May 15	8	34.5	40.5
	3	May 1–May 29	May 15–June 12	9.5	28	35
	4	May 29–July 3	June 12–July 3	10.5	28	32.5
	5	July 3–July 24	July 3–July 24	11.5	21	30
	6	July 24–Aug. 14	July 24–Aug. 14	12.5	21	27.5
C. finmarchicus	2[b]	May 29–July 3	May 22–July 3	10	45.5	46.5
	3	July 3–Aug. 14	July 3–Aug. 21	11.5	45.5	41
T. longicornis	2	Apr. 10–May 15	Apr. 10–May 22	8.5	39	41
	3	May 15–June 19	missing data	10.5	35	33.5
	4	June 19–July 24	missing data	12.5	35	27.5
	5	July 24–Aug. 28	July 17–Aug. 21	13.5	35	25.5
A. clausi	2	Apr. 10–May 22	missing data	8.5	42	35.5
	3	May 22–June 26	May 29–June 26	11	31.5	27.5
	4	June 26–July 31	June 26–July 24	11	31.5	27.5
	5	July 31–Aug. 28	no size change	13.5	28	22
O. similis	2	Apr. 17–June 19	Apr. 24–July 3	9	67	57
	3	June 19–July 31	July 3–Aug. 7	12	39	37.5
	4	July 31–Sept. 11	no size change	13.5	42	31.5

[a]C IV used for *P. minutus*.
[b]G_2 is assumed to have started from the second period of maturation of G_1 which was followed by successful spawning (see text).

It can hardly be doubted that *P. minutus* (Fig. 2) exhibited a succession of six generations (and possibly later ones) between spring and late summer, as summarized in Table 1. However, throughout the latter part of the summer there was an accumulation of C IV. These animals were, as Marshall (1949) concluded, in arrested development, in preparation for overwintering. At the same time a smaller fraction of the population continued to mature through successive generations.

Pseudocalanus has been reared in a number of laboratories. Paffenhöfer and Harris (1976) reared North Sea animals to maturity at 12.5°C. Only at the lowest food level used ($25\ \mu g\ C \cdot L^{-1}$) was there evident retardation of development. At higher food levels they found that "generation times" (not defined, but presumably egg to egg) in seven experiments averaged 27.7 d. A temperature function (McLaren 1966) for embryonic duration of *Pseudocalanus* from Millport, near Loch Striven, is

$$D = 536(T + 9.99)^{-1.68},$$

giving

$$D = 5174(T + 9.99)^{-1.68}$$

for generation length (Table 1). Alternatively, use can be made of data from extensive rearings of North Sea animals at a number of temperatures by Thompson (1976). These were analyzed by Corkett and McLaren (1978), who fitted $D = 20025(T + 11.45)^{-2.05}$ for the period between egg laying and maturity. This yields 29.8 d at 12.5°C, perhaps reflecting mild retardation compared with the findings of Paffenhöfer and Harris (1976), and estimated generation lengths are about 5 d longer at 8°C and 2 d longer at 12.5°C than those used here (Table 1). Finally, as a demonstration that predictions may be made from geographically remote populations, the temperature function for animals from Nova Scotia (McLaren 1974) for the period from egg laying to mature female,

$$D = 21494(T + 13.4)^{-2.05},$$

gives virtually identical predictions of generation lengths to those used here (Table 1).

The predicted generation lengths are consistently longer than those inferred from samples (Table 1). Possibly animals, especially later in the season when discrepancies are greatest, migrated into warmer surface waters at night. Alternatively, the small proportion of the population that matured later in the season (Fig. 2) could have lived nearer to the surface. Corkett and McLaren (1978) reviewed the tendency of resting stages of *Pseudocalanus* to be found in deeper, colder water. They also showed that observed generation lengths in Loch Striven were indeed more closely predicted from surface temperatures (from Thompson's 1976 data; also using hatching to maturity as a "vanguard" estimate — see Materials and Methods).

The important conclusion here is that generations of *P. minutus* in Loch Striven were, if anything, shorter than predicted from temperatures during the long period from spring to late summer. In addition, although it cannot be determined precisely, Marshall (1949) suggests that the "first brood" (G_0 on Fig. 2) was spawned

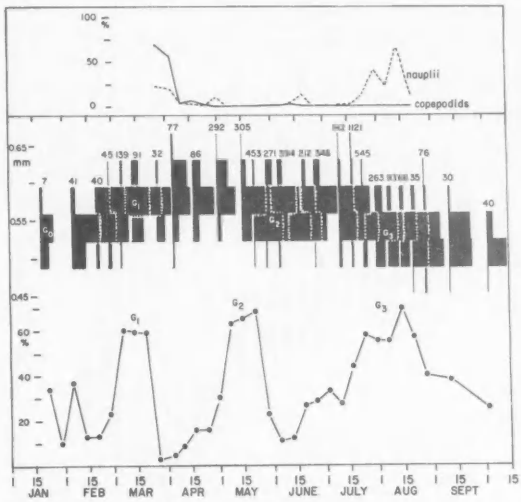

FIG. 3. Life cycles of *Microcalanus pygmaeus* in Loch Striven. See Fig. 2 caption. Data from Marshall (1949, tables V and XII).

largely around February 20, about 42 d before it matured (April 4, Fig. 2); the predicted generation length at 7°C (Fig. 1) is 44 d.

It thus appears that generation length in *P. minutus* was unaffected by food supply from late winter to late summer, at least. There is no reason to suppose that the population increasingly entered a resting stage in summer to escape immediate food shortage. On the contrary, larger, more oil-rich (i.e. obviously well-fed) animals from Nova Scotia are more prone to enter a resting stage in C V, and this may be partly under genetic control (McLaren and Corkett 1978).

Microcalanus pygmaeus

Marshall (1949) concluded that numerous nauplii on January 17 "grew up to adults during February and March," and that "the second brood . . . reached the adult stage in the beginning of May." She did not make explicit conclusions about subsequent generations. Analysis of C V cohorts and female size (Fig. 3) indicates more precisely that G_1 matured mostly in mid-March and G_2 mostly in mid-May. Marshall was impressed with the large increases in numbers of adults in early May (Fig. 3), but these did not herald G_2, and may have been due to sampling variability or patchiness (see Materials and Methods). Size variation from May through July was quite unlike the pattern found in any other species discussed in this paper. The mode of size alternated week by week on either side of 0.55 mm. This could not have reflected rapid turnover of the female population. It could have resulted either from fine-scale variability in relation to sampling periodicity (tidal, or depth of sampling in a deep, stratified population?), or from varying bias in the measurement of individuals very close to 0.55 mm. It is clear that a real change in adult females, consistent with the CV cohort, occurred when individuals of ca. 0.51 mm became common and the mode at ca. 0.57 mm no longer recurred (Fig. 3).

Microcalanus pygmaeus seems to have had longer generations than any other species in Loch Striven, about 77 d for both G_2 and G_3 (from intervals between peaks of C V and size changes on Fig. 3). Since temperatures increased with season, it seems that G_3 was retarded relative to G_2; this also seems to be indicated by the "hesitant" establishment of the CV cohort of G_3. Unlike any other species in Loch Striven, *M. pygmaeus* spent virtually all its time below 10 m, where it may not have depended on phytoplankton (Marshall 1949). Apparently it has not been reared in the laboratory.

Calanus finmarchicus

Marshall et al. (1934) interpreted the life cycle of this species in Loch Striven with confidence. They concluded that overwintered C V matured through January and February and gave rise to the first generation, only a small portion of which matured and spawned, unsuccessfully, in April. Most of this first generation remained as copepodids, which matured around May 22 to produce the "true" second generation, which in turn spawned the third generation in July. Most of these formed the overwintering stock of C V, but a few matured and spawned in August and September. The first appearance of large females on April 10 (Fig. 4) is clearly the unsuccessful "brood" of Marshall et al., referred to above. The rest of G_1 stayed in C V, which also continued to accumulate more younger animals during April and May. These C V matured in numbers that exceeded those that matured earlier by May 22, which can be taken as the time of origin of G_2 based on size changes. Thereafter, both size changes and cohort peaks were clear (Table 1).

Curiously, there seem to be no published accounts of laboratory rearings of *C. finmarchicus* subsequent to the pioneer efforts of Nicholls (1933), which were analyzed further by Marshall and Orr (1955). Although conditions of rearing were not carefully controlled, water was changed daily to provide fresh, natural food; temperatures were about 14–15°C when measured. Nicholls added minimum times taken by different stages in a series of experiments to conclude that 27 d were required from egg laying to maturity. However, some of his estimates were biased and others included guesswork. Marshall and Orr (1955) accordingly singled out three adult females from the only family for which the period from egg laying to C I was actually known by Nicholls. These individuals took 31, 32, and 35 d, respectively, to reach maturity from the egg. Using the temperature function for embryonic duration of *C. finmarchicus* from nearby Millport (McLaren

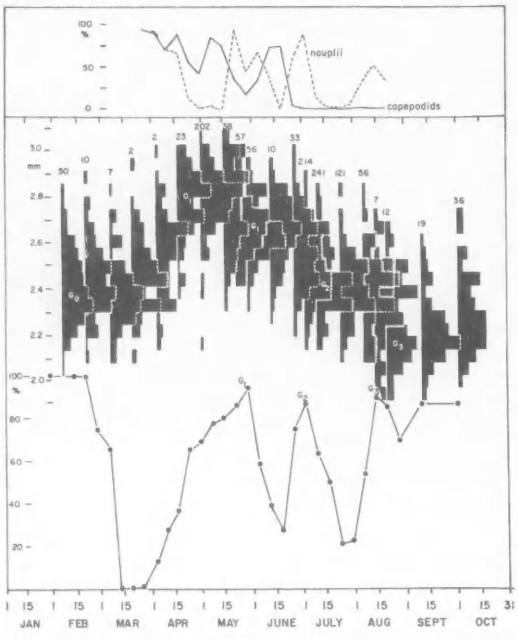

FIG. 4. Life cycles of *Calanus finmarchicus* in Loch Striven. See Fig. 2 caption. Data from Marshall et al. (1934, plate II and tables I and II).

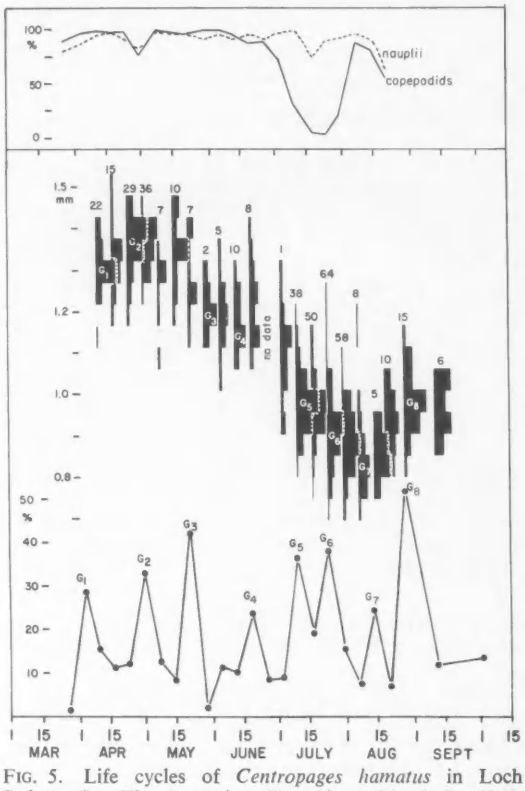

FIG. 5. Life cycles of *Centropages hamatus* in Loch Striven. See Fig. 2 caption. Data from Marshall (1949, tables VI and XIII).

1966) and taking 32.7 d as an estimate of mean generation length at 14.5°C gives

$$D = 6779(T + 9.43)^{-1.68}.$$

Agreement of predicted with inferred generation lengths is good (Table 1). Use of a function for embryonic duration of animals from Norway (McLaren et al. 1969) giving

$$D = 31630(T + 14.1)^{-2.05},$$

produces almost identical results.

It cannot be assumed that the "stalling" of G_1 during April and May was due to insufficient food to allow animals to mature, for at the same time animals continued to accumulate as C V. This delay could have been adaptive, the animals arresting their development in C V because food was not appropriate or adequate for potential offspring. At any rate, food could not have been seriously restrictive after late May, and the bulk of animals that failed to develop further after early August were in a resting stage, preparatory for winter. Only a small portion, not necessarily the best fed (see under *P. minutus*), "chose" to reach maturity at a temperature-dependent rate.

Centropages hamatus

Marshall (1949) concluded that the life cycle of this species began with a first "brood" (generation) maturing in April, showed a second about May 22, a third in June, and a fourth in July. Analysis of her data (Fig. 5) suggests that generations were more frequent than she supposed, exceeding even *P. minutus* (cf. Fig. 2). Resting, overwintering eggs are known in *C. hamatus* (Pertsova 1974). The slightly smaller females of G_1 might have developed in part when waters were actually warmer than in April (Fig. 1). The frequent coincidence of peaks in the C V cohort with changes in female size (which sometimes even preceded the peaks in C V) is what one would expect with such a rapid series of generations. Errors from gaps in the sampling schedules are more critical when generations are short, but it seems that these were about 25 d in the first half of the season, and 20 d in the second half.

Only Pertsova (1974) appears to have reared *C. hamatus* in the laboratory. She found that unfed nauplii from the White Sea took about 1.5 and 2 d at 12–13°C to pass through N I and N II, respectively, but did not survive N III (probably the first feeding stage). Evidently by extrapolation (i.e. assuming isochronal development) she suggested 10–12 d for naupliar development at 12–13°C. Summer (nonresting) eggs were observed to take about a day to hatch at the same tem-

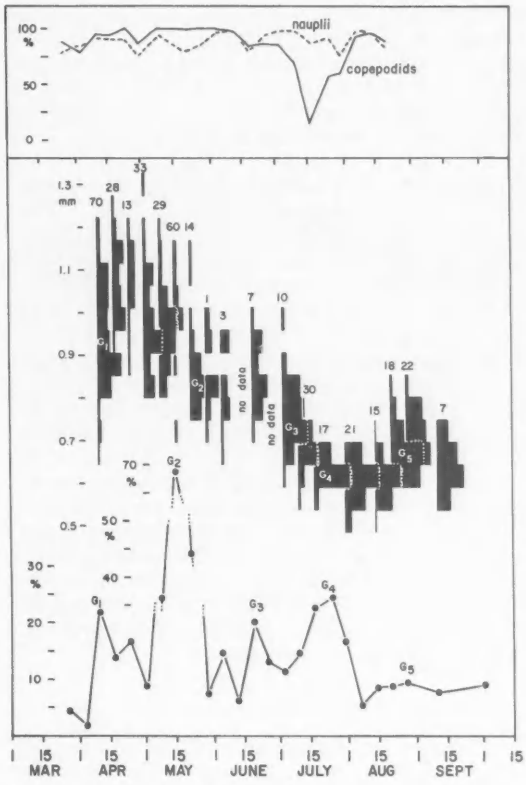

FIG. 6. Life cycles of *Temora longicornis* in Loch Striven. See Fig. 2 caption. Data from Marshall (1949, tables VII and XIV).

perature. Pertsova also reared animals in containers suspended in the sea, but does not explicitly record food levels and temperatures. She states, without details, that duration of the copepodid period at 12–13°C was 18–20 d, and that overall development time from egg laying to adult is 28–32 d. The length of the second generation in the White Sea during July and August 1969 and 1971 was found from clearly depicted cohorts of eggs and other stages to be about 1 mo. She claims that this matched her laboratory estimates, since the surface waters where the copepods lived were about 12–13°C at the time (no data given). However, elsewhere (in relation to body size) she tabulates temperatures of 2.5–4.2°C for the period of June 27 to July 10, 1971, and 10.7°C for August 24, 1971. It seems that the copepodid period estimated by Pertsova is too long. If isochronal development applies, then the copepodid period should also be 10–12 d, and (including embryonic duration) generation length of food-satiated animals at 12–13°C should be 21–25 d. This estimate is in keeping with those from Loch Striven and also supports the above inference from Pertsova's work that animals in the White Sea were in fact experiencing temperatures lower than 12–13°C.

Temora longicornis

Populations of this species in Loch Striven in January and February consisted almost entirely of nauplii, and Marshall (1949) concluded either that there is a resting egg in this species (confirmed by Pertsova 1974) or that adults overwintered out of the reach of nets (unlikely). She believed that the first generation of large adults appeared in April, and the second on about May 10. She noted that "another great increase of nauplii (on July 10) coincided with the next diatom increase, but apparently no brood developed from these nauplii." She also felt that "there was no distinct autumn brood." Marshall's assessment of G_1 is supported by the C V cohort (Fig. 6). Although there was some subsequent instability of size modes, a sharper change occurred after the passage of the very prominent peak of C V, representing G_2. Later, G_3, G_4, and G_5 seemed well defined, in spite of small samples and lack of data at times in June. The increase in size of females in G_5 over those in G_4 is marked, although it might be argued from depth distributions (Fig. 6 top) that G_5 developed in warmer water (Fig. 1).

Harris and Paffenhöfer (1976) give a mean "generation time" (see under *P. minutus*) of 27.7 d for *T. longicornis* from the North Sea reared at 12.5°C in 10 experiments, at food levels evidently adequate for full (virtually isochronal) development rate. McLaren et al. (1969) gave embryonic duration for animals from Nova Scotia as

$$D = 1346(T + 10.4)^{-2.05}.$$

Corkett and McLaren (1970) used this function to describe the period from hatching to C I as

$$D = 8313(T + 10.4)^{-2.05},$$

which predicts 13.6 d at 12.5°C compared with a mean of 12.6 d found by Harris and Paffenhöfer (1976). Thus the same function fitted to their estimate of 27.7 d at 12.5°C gives

$$D = 16988(T + 10.4)^{-2.05}$$

to predict food-satiated generation lengths. These match generation lengths inferred from samples (Table 1). The apparent retardation in G_5 may not be real, as size changes (see above) imply that these animals may actually have developed at colder temperatures than did those of G_4.

Acartia clausi

Marshall (1949) concluded that the first generation developed in the latter half of March, although numbers were low, that a second generation matured at the beginning of May, a third during June, and a fourth at

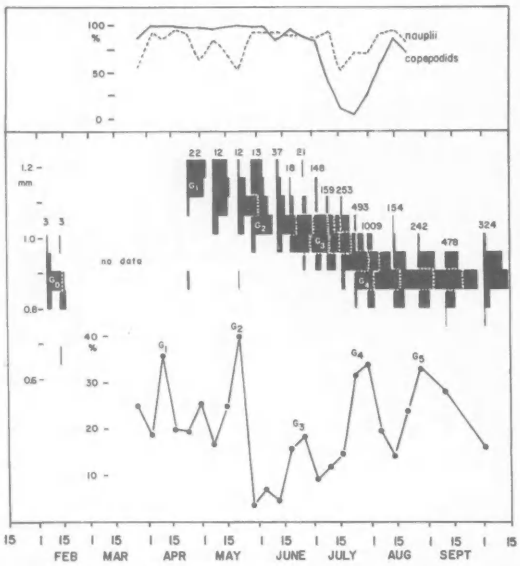

FIG. 7. Life Cycles of *Acartia clausi* in Loch Striven. See Fig. 2 caption. Data from Marshall (1949, tables VIII and XV).

the end of July and beginning of August. She felt that "after this, breeding seemed to be continuous." Except for her supposition that the second generation matured in early rather than late May, analysis of C V cohorts and female sizes (Fig. 7) broadly supports Marshall's conclusions, but allows more precise estimates of generation times (Table 1).

Only Landry (1975) has given an extensive account of precise development times at high food levels for animals from any locality. His data from animals off the coast of Washington were used by Miller et al. (1977) as a prime example of isochronal development (see Materials and Methods). Fitting to Landry's (1975, his table 1) data the equations

$$D = 1442(T + 10.49)^{-2.05}$$

for embryonic duration and

$$D = 6866(T + 10.49)^{-2.05}$$

for hatching to C I, and assuming isochronal growth for missing data at 10°C and 15°C, gives

$$D = 14748(T + 10.49)^{-2.05}$$

for length of a generation (egg laying to maturity).

Predictions from these equations can be compared with limited laboratory data from other localities. There seem to be no rearings of *A. clausi* from the western North Atlantic other than those by Corkett (1968). His figure 4 suggests a time between generations of animals from Plymouth, England, of roughly 25 d at 14–16°C without close control of food. Predicted length is about 19.3 d. Sazhina (1974) summarizes work on Black Sea animals and gives 10 d for hatching to C I at 14–16°C (predicted 9.0 d at 15°C) and 11.5 d at 20–22°C (predicted 5.8 d at 21°C). Sazhina's estimates do not reflect the expected negative effect of temperature, and furthermore, an earlier report (Sazhina 1968) gives 18–21 d for C I to maturity at 20–22°C, clearly not isochronal. Probably Sazhina's estimates are not food-satiated development rates, at least at 20–22°C. Corkett and McLaren (1970) give a mean of 9 d at 12.9°C (predicted 10.7 d) for four animals from Nova Scotia reared from hatching to C I. Iwasaki et al. (1977) tabulate development times from egg hatching to egg laying (perhaps close to the period that is taken here as generation length) of animals from Japan as 19.4 d at 15°C (predicted 19.3 d) and 16.7 d at 20°C (predicted 13.4 d). Perhaps, as suggested more strongly for the Black Sea animals, development was retarded at 20°C.

Agreement between predicted generation lengths and those estimated from samples from Loch Striven is quite good (Table 1). The most divergent prediction, in late summer, may indicate that the animals of G_5 in fact did not, as inferred from approximate depth distributions (and Fig. 1), experience higher temperatures than did those of G_4; the lack of size difference between G_4 and G_5 (Fig. 7) also suggests this.

Oithona similis

This was the most abundant small copepod in Loch Striven (Marshall 1949). In January the stock consisted mostly of adults and their nauplii. Marshall concluded that a rise in relative abundance of nauplii in late March (coincident with a diatom bloom) gave rise to a generation maturing at the end of April. She thought that then "the adults immediately produced another brood which grew up during May, but after this breeding was continuous." Her data (Fig. 8) suggest that Marshall was misled by the burst of nauplii in late March (a possible net-clogging error; see *Materials and Methods*), and that G_1 must have originated earlier. However, Fig. 8 shows that G_1 matured in April, that G_2 "grew up" during May, and clarifies subsequent events. The C V cohort of G_3 appears untrustworthy, but the distinct drop in female size that followed the main peak is unambiguous. The dating of G_4 from its C V cohort is imprecise without samples from the preceding and following weeks. The estimated generation lengths (Table 1) are among the longest of any species in Loch Striven.

Eaton (1971) reared *O. similis* from Nova Scotia in defined food conditions that she believed allowed full development rates of nauplii. She found that at 10°C animals averaged 21.0–25.3 d (overall mean 23.4 d) from hatching to C I in four experiments with a variety of cryptophyte flagellates as food. After C I the animals became unhealthy and retarded, and she suggested that at the earlier rate (i.e. isochronal development), ani-

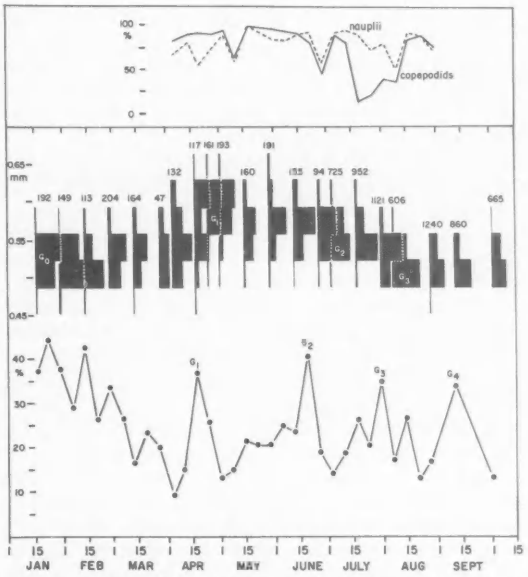

FIG. 8. Life cycles of *Oithona similis* in Loch Striven. See Fig. 2 caption. Data from Marshall (1949, tables IX and XVI).

mals might have matured at 1.4–1.6 mo. She also measured embryonic durations accurately, and described them by

$$D = 1430(T + 5)^{-2.05}.$$

Fitting this to the 23.4-d naupliar period at 10°C yields

$$D = 6028(T + 5)^{-2.05}.$$

Assuming isochronal growth (five more stages of equal duration added to the six from hatching to C I) gives

$$D = 11052(T + 5)^{-2.05},$$

and generation length (by adding embryonic duration) is

$$D = 12482(T + 5)^{-2.05}.$$

Sazhina (1974) gives 15–19 d for the period between hatching and C I of *O. similis* in the Black Sea at 8–10°C. This does not agree with the above estimates for animals from Nova Scotia and suggests that perhaps the experiments cited by Sazhina were not as carefully controlled, or that there is a geographical variation.

In spite of the several assumptions, generation lengths inferred from samples from Loch Striven are close to those predicted from experiments in Nova Scotia (Table 1). As noted above, the time of the C V cohort of G_4 is more approximate than that of other peaks. Also, the fact that size did not change (Fig. 8) suggests that the animals in G_4 did not experience the higher temperatures assumed in Table 1.

Discussion

Much of the foregoing has of necessity been based on techniques for the detection of successive generations of copepods from samples. The general concordance of conclusions from copepodid cohorts and female sizes has, hopefully, been doubly convincing. However, the main intent has been to promote ways of estimating demographic and production parameters without the tedium of such techniques, and even without the degree of life-cycle synchrony that is evident in enclosed waters like Loch Striven. The analyses assumed that development rates in nature were uninfluenced by variations in food level and predictable from temperatures alone. The match between observations and predictions (Table 1) is surely as good as could be expected from data obtained from such disparate sources in the literature. Most predicted generation lengths are slightly shorter than those inferred from samples. Perhaps the assumption (see *Materials and Methods*) that animals routinely produce eggs immediately upon maturing does not hold for most species.

In particular, the results seem to support the adequacy of Bělehrádek's temperature function for description of durations of stages and generation lengths. The results also imply that the parameters of this equation may vary little within species in various parts of their ranges. However, the precise form of Bělehrádek's function (criticized, for example, by Bottrell 1975 as marginally less precise statistically than a log-quadratic equation) matters little over the temperature ranges in Loch Striven. Over wider temperature ranges, the form of function may be more important. For example, recently Razouls (1974) used a power function,

$$D = a \cdot b^T,$$

to relate temperature (T) and generation length (D), estimated from cohorts, of two Mediterranean copepods. Both his plots of log D against T show a mild concavity that would be corrected by Bělehrádek's function.

Bělehrádek's function is also empirically convenient in requiring a change only in the parameter a, where different stages occupy fixed proportions of total development time at all temperatures. The successful application of this rule in the present paper should again not be taken as a detailed validation of the function, considering the small temperature ranges involved. However, assuming isochronal development involves more precise predictions, and the present study tends to validate this rule and indicate that it applies more generally than Miller et al. (1977) supposed. Of interest in this context is the summary by Gaudy (1976, table II) of available laboratory estimates of naupliar and copepodid periods of five species of Mediterranean copepods. For each of the five species, some authors had demonstrated isochronal or near-isochronal development, while others had found retarded copepodid development. Doubt may be expressed about adequacy of food in laboratory situations when the copepodid period

much exceeds that for nauplii. Furthermore, the present results suggest that isochronal development should be kept in mind as an expectation when interpreting samples from reasonably productive marine waters. For example, Gaudy (1976) traced cohorts (each copepodid and adult stage expressed as a percentage of total numbers) by eye-fitted lines that always depict increasing duration of later stages. Partly this is because he included adults; these persist through longer time periods than do copepodids, so that peaks in adult abundance are misleading (discussed in Materials and Methods). Among the five species, Gaudy included *A. clausi*, and concluded that it had a naupliar period of 10 d and, from cohorts, a copepodid period of 17.5 d. A line drawn only through copepodid peaks (his figure 4) gives an estimated copepodid period of 12 d, more nearly isochronal with the naupliar period.

Of course, isochronal development may not occur in the sea at high latitudes (e.g. McLaren 1969) and in some temperate freshwaters (e.g. Rigler and Cooley 1974). The retardation of development of older stages in such situations may indeed be a result of food shortages. However, the possibility remains that some retardation is "voluntary," like the resting stages in Loch Striven, as an adaptation to attune life cycles, especially annual ones, to seasonal constraints.

The usefulness of the above functions and rules suggests that attention should be given to other possible predictive approaches. Apparently no one has attempted to test or extend the relationship described by Corkett and McLaren (1970), whereby development times of older stages might be estimated from embryonic duration, together with the relative linear dimensions of eggs and bodies of copepods, although Alcaraz (1977) has used the relationship in an unverified attempt to predict the duration of the naupliar period in two species of *Acartia* from Spain. Another promising rule has been promoted by Ivanova (1974) and by Miller et al. (1977), namely that copepods may increase weight between molts by a constant fraction (Brook's Law, a linear increase of 25% or a doubling of weight between molts, is a special case). Together with isochronal development, this implies that growth in weight may be exponential in time, at least when temperatures are constant. This would be of great convenience in production studies, for then P/B ratios would be the same for all stages when development is uninhibited by food shortages. The seasonal effects of temperature on size (McLaren 1963; Miller et al. 1977) and development times (above) might readily be incorporated.

All these potentially useful rules may ultimately be reduced to measurable differences at the cellular level. Woods (1969) found marked differences in DNA content of embryonic nuclei in two cryptic species of *Pseudocalanus* from arctic Canada. She suggested that nucleus size controlled cell size and division rate, which in turn governed the differences between the two species in body size and development rates. McLaren (1976b) showed a similar correlation between body size and DNA content of somatic nuclei of adult females of *Pseudocalanus* from Nova Scotia. Alcaraz (1977) found that adults of *Acartia clausi* and *A. margalefi* in Spain had suggestively similar estimated ratios of carbon contents (4.62 vs. 1.20 μg) and somatic nucleus volumes (1.85 vs. 0.73 μm^3).

The present study may also have wider significance for current fashions in biological oceanography. There is an enormous literature on feeding physiology and trophic ecology of marine copepods. However, the present study suggests that if a variety of copepods in a not particularly rich temperate environment passed through successive generations at rates that were unaffected by food, then studies of their food and feeding would not tell us much about what governed their life histories and contributions to secondary production. This is reinforced by laboratory studies of some species showing that body weights (e.g. Harris and Paffenhöfer 1976; Paffenhöfer and Harris 1976) and rate of egg production (e.g. Corkett and McLaren 1978) may also be little affected by food levels higher than those permitting full development rates. Rather, it seems that more study should be made of the "intrinsic" adaptations that determine performances of copepod species in nature. Why do *P. minutus* and *C. finmarchicus* appear to "give up" and enter resting copepodid stages at times when food may still be available for sustained development and production? Do other species that produce resting eggs similarly anticipate food shortage rather than actually experience it? What are the adaptations and mechanisms underlying the fact that the two smallest species in Loch Striven, *M. pygmaeus* and *O. similis*, appear to have the longest generation lengths? What is the nature of *C. hamatus* that allows it to have the shortest generations and therefore possibly a relatively high rate of production and use of resources?

Finally, there may be lessons in Loch Striven for those interested in interactions among species and the structure of plankton communities. Recently Steele and Frost (1977) constructed a simulation model of the dynamics of *C. finmarchicus* and *P. minutus* in Loch Striven and the interactive impact of these copepods on the abundance and size structure of the phytoplankton. The simulated populations, unlike those in nature, collapsed after a month or so, perhaps, according to the authors, because too little capacity for nutrient regeneration had been built into their model. But, perhaps as important as the specific failing of their model is the assumption that the system had to be analyzed and understood in terms of such interactions. More generally, there is a growing literature applying the paradigms of competition and niche theory to zooplankton communities (e.g. review by Hall et al. 1976), and some insist that aquatic communities cannot be understood without the utmost attention to such interactions (Lane and Levins 1977). From the present analysis it seems that intraspecific and interspecific competition for resources need not be important to marine copepods. Copepodologists might also bear in mind that the ele-

gant and seemingly persuasive developments of niche theory and community dynamics have arisen out of largely terrestrial studies that may rest on dubious empirical support (Wiens 1977).

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Application of Antibiotics in the Cultivation of Dungeness Crab, *Cancer magister*

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FISHER, W. S., AND R. T. NELSON, 1978. Application of antibiotics in the cultivation of Dungeness crab, *Cancer magister*. J. Fish. Res. Board Can. 35: 1343–1349.

In static cultivation of *Cancer magister* zoeae, duration of antibiotic treatment, effectiveness of various antibiotics, and therapeutic pretreatment of the live food were evaluated. Periodic treatment with antibiotics was necessary to maintain high zoeal survival, the greatest benefit occurring during the early zoeal period. Low concentrations (1–10 mg/L) of chloramphenicol were equally or more successful than 100 mg/L streptomycin plus 100 mg/L penicillin. Kanamycin (100 mg/L) and neomycin (50 mg/L) appeared toxic to the zoeae. Successful antibiotic treatment did not alter the development and increased the weight of cultivated zoeae, although these were all lighter than captured crabs of the same apparent age. There was indication that elimination of bacterial epibionts with antibiotics restricted the attachment of other microbial epibionts.

Key words: aquaculture, mariculture, epibionts, Crustacea epibionts, zoeal survival, *Artemia salina*, antibiotics

FISHER, W. S., AND R. T. NELSON, 1978. Application of antibiotics in the cultivation of Dungeness crab, *Cancer magister*. J. Fish. Res. Board Can. 35: 1343–1349.

Nous avons évalué la durée du traitement aux antibiotiques et l'efficacité de divers antibiotiques et du prétraitement thérapeutique de la nourriture au cours d'expériences de culture en milieu statique de zoés de *Cancer magister*. Le traitement periodique aux antibiotiques est nécessaire au maintien d'une bonne survie des zoés, les meilleurs résultats étant obtenus au début du stade zoé. De faibles teneurs (1–10 mg/L) de chloroamphénicole sont tout aussi efficaces, ou même davantage, que 100 mg/L de streptomycine + 100 mg/L de pénicilline. La kanamycine (100 mg/L) et la néomycine (50 mg/L) semblent toxiques pour les zoés. Un traitement réussi aux antibiotiques n'affecte pas le développement et contribue à une augmentation de poids des zoés cultivés, bien que ceux-ci soient tous plus légers que des crabes capturés dans la nature et de même âge apparent. Il ne semble pas que l'élimination d'épibiontes bactériens à l'aide d'antibiotiques restreigne la fixation d'autres épibiontes microbiens.

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LABORATORY cultivation of Dungeness crab (*Cancer magister*) zoeae was first reported by Poole (1966) who used antibiotics in the culture water to maintain zoeal survival. Concentrations of 132 mg/L streptomycin plus 8.3 mg/L penicillin were added to the culture water which was changed every other day. Poole noted that with antibiotic treatment, "clinging particles, presumed to be bacteria, began to disappear from appendages and spines of infected larvae." Fisher and Nelson (1977) showed recently that *C. magister* zoeae exhibit increased survival in response to increased additions of streptomycin and penicillin with water changes and antibiotic renewal 3 times weekly. Reed (1969), however, attained 68–88% zoeal survival without antibiotics while studying the effects of temperature and salinity. Glimme (1974) did not use antibiotics and found bacterial infestation to correspond with high zoeal mortality, while Buchanan et al. (1970) had no difficulty rearing untreated zoeae. Despite contradicting evidence for Dungeness crab larvae, microbial epibionts have often been held responsible for egg and larval mortalities of cultured marine Crustacea (reviewed by Fisher 1977).

D'Agostino (1975) has shown several antibiotics to be more successful in controlling marine bacteria than streptomycin and penicillin and has demonstrated tolerable levels of these antibiotics for treatment of *Artemia salina* nauplii and adults. These levels of antibiotics are useful guidelines for antibiotic treatment for other marine crustaceans, although screening for activity against local flora is necessary.

Glimme (1974) and Fisher and Nelson (1977) have indicated that feeding live *A. salina* nauplii could be partially responsible for the introduction of bacteria that ultimately cause zoeal mortalities. The reduction of these bacteria by pretreatment *A. salina* nauplii should enhance zoeal survival and might partially replace the need for direct antibiotic treatment.

In an effort to determine the most productive methods for utilizing antibiotics in Dungeness crab cultivation,

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we measured crab zoeae survival in relation to different durations of antibiotic treatment, different types and concentrations of antibiotics, and therapeutic pretreatment of the live food, *A. salina*.

Materials and Methods

Variable Duration of Antibiotic Treatment

An ovigerous Dungeness female was caught in a trap near Fort Bragg, Calif., on February 5, 1977, and held in the nearby Noyo River for 2 h, then packed in wet seaweed and transported by automobile to Bodega Marine Laboratory, Bodega Bay, Calif. In the laboratory, the crab was held in a 110-L sand substrate aquarium at ambient temperatures. Eggs began to hatch on February 7, 1977, and zoeae were removed from the aquarium daily. Those that hatched on February 9 were held for 2 d without food and then 50 were transferred into each of the lidded, glass test jars which held 1.5 L of seawater and were placed in a cold room at 12°C. The seawater was filtered (5 μm) by resin cartridge filters and sterilized by ultraviolet irradiation. The crab zoeae were counted and transferred to fresh seawater every other day throughout the experiment. They were fed *A. salina* nauplii at a concentration of 3/mL with each water change until 60 d old when the concentration was increased to 6/mL for the remainder of the experiment.

The antibiotics streptomycin sulfate and penicillin-G were added to specific test jars at a concentration of 100 mg/L according to the following schemes: a) none, b) every water change, c) every second water change, d) every fourth water change, e) the first 2 water changes, f) the first 5 water changes, and g) the first 10 water changes. Each of these conditions was carried out in duplicate and light:dark periods were maintained at approximately 14:10.

As the zoeae became megalops, they were counted and removed from the test jars daily for placement in a flow-through seawater system, where they were fed live adult *A. salina*. Ten megalops (1–2 d old) cultured with antibiotics were placed in 95% ethanol for 3–5 min to reduce salt adherance, rinsed in glass distilled water for 2 min, blotted, desiccated for 2 h at 60°C, and individually weighed. Similarly, 10 megalops cultured with antibiotics and four untreated cultured megalops were weighed 1–2 d prior to becoming first instars and wet weights were obtained before desiccation. Ten antibiotic-treated crabs were weighed 5 d after becoming first instars. For comparison, 20 megalops from nature were weighed 1–2 d before becoming first instars and 10 crabs were weighed 5 d after becoming first instars. These crabs were captured in the Bodega Harbor, Bodega Bay, Calif., on April 10–12, 1977, and several were metamorphosing at that time. They were held in the laboratory about 1 wk until the final weighing as 5-d instars.

Comparison of Various Types and Concentrations of Antibiotics

Antibiotic types and concentrations were tested against microorganisms from the crab culture system in the following manner. Synthetic marine broth (Fisher et al. 1976) was inoculated with crab culture water and incubated for 6 h at 30°C. One milliltre of this turbid culture was added to a sterile 1% synthetic marine broth solution with test antibiotics and brought to a final volume of 100 mL with filtered, autoclaved seawater. Samples were taken aseptically for photometric analysis on the Spectronic 20 (Bausch and Lomb) and discarded. In the initial experiments cultures were sampled 3 times within 6 d and were measured at 475 nm. In addition to an untreated control, the antibiotics tested were 1, 10, 50, and 100 mg/L neomycin; 1, 10, 50, and 100 mg/L kanamycin; and 0.1, 1, 10, and 50 mg/L chloramphenicol. A final screening was also conducted in the same manner to refine concentration effects and to test certain antibiotics in combination. These tests included an untreated control; 1, 2.5, 5, and 7.5 mg/L chloramphenicol; 10 mg/L streptomycin; 100 mg/L penicillin; 100 mg/L kanamycin; 50 mg/L neomycin; 100 mg/L streptomycin plus 100 mg/L penicillin; 1 mg/L chloramphenicol plus 100 mg/L kanamycin; 1 mg/L chloramphenicol plus 50 mg/L neomycin; and 100 mg/L kanamycin plus 50 mg/L neomycin. These were aseptically sampled every day for 4 d and measured at 610 nm on the spectrophotometer.

The most effective combinations and concentrations of antibiotics determined from the screening process were then tested on cultivated crab zoeae. A female crab, trapped off the Russian River (Jenner, Calif.) in November 1976 was held at the laboratory in a communal holding tank with 12 cm sand substrate and raw flow-through seawater. She extruded eggs just prior to February 14, 1977. She was maintained in the flow-through system until May 27, 1977, and then moved into a 12°C cold room in a 110-L aquarium with 40 L of filtered, ultraviolet irradiated seawater. An airstone was used for aeration until the eggs began to hatch on May 29, 1977. The zoeae were removed from the aquarium daily. Those that hatched on May 31 were held unfed for 2 d and then 50 were distributed into each of the glass test jars holding 1.5 L of filtered, sterilized seawater. These tests included an untreated control; 1, 5, and 10 mg/L chloramphenicol; 100 mg/L kanamycin; 50 mg/L neomycin; 100 mg/L streptomycin plus 100 mg/L penicillin; 1 mg/L chloramphenicol plus 50 mg/L neomycin; and 1 mg/L chloramphenicol plus 100 mg/L kanamycin. Three replicates of each condition were performed and the test jars were covered and maintained in the 12°C cold room. Zoeae were fed live *A. salina* nauplii at a concentration of 3/mL with each water change until 30 d old when the experiment was terminated. The water was changed and the food and antibiotics were replenished twice each week during this period.

Therapeutic Pretreatment of Live Food

Artemia salina nauplii were separated, counted, and divided into two flasks, each containing 1 L of seawater at a naupliar concentration of 15/mL. One of the flasks was treated with 100 mg/L streptomycin plus 100 mg/L penicillin while the other was treated at 10 mg/L malachite green. Aliquots of 200 mL were taken from each flask after 0, 2, 4, and 6 h of treatment at room temperature. These were screened in a plastic mesh, rinsed in filtered ultraviolet irradiated seawater for 2 min, resuspended in 1 L of seawater, and placed in the 12°C cold room at a naupliar concentration of three per milliltre. A flask of 200 mL seawater without *A. salina* napulii was treated similarly as a contaminant control. Water samples were obtained from the cold room aliquots 24, 48, and 72 h after the treatment for each had been concluded. The samples were serially diluted and spread on synthetic marine agar plates (Fisher et al. 1976). Bacterial colony counts were made after 24-h incubation at 25°C.

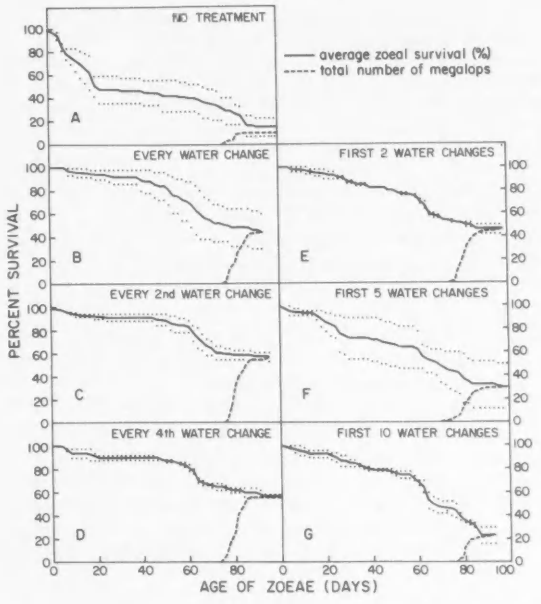

FIG. 1. Crab survival results with various antibiotic treatment schedules for 100 d using streptomycin and penicillin at 100 mg/L each. Solid lines represent averages of the points shown for two replicates; dashed lines show number of megalops produced from both replicates.

Similarly treated *A. salina* nauplii were fed to crab zoeae in culture. An ovigerous female crab was captured off the Russian River, Calif., on June 5, 1977, and was brought to the Bodega Marine Laboratory where she was held in a 100-L plastic trough with 1 μm filtered, ultraviolet irradiated seawater at 12–13°C and 5 cm sand substrate. Zoeae used in the experiment were hatched on June 29, and held unfed for 2 d before being transferred to the glass test jars. Fifty zoeae were placed in each test jar containing 1.5 L of filtered, ultraviolet irradiated seawater and held in a 14°C cold room. Three replicates were run for each of the four combinations of the following conditions: 1) no treatment to *A. salina* nauplii vs. pretreatment for 2 h with 10 mg/L chloramphenicol and 2) no treatment of *C. magister* zoeae vs. treatment with 100 mg/L streptomycin plus 100 mg/L penicillin with each water change. *Artemia salina* nauplii were treated, screened, and rinsed as described earlier. Treated or untreated, *A. salina* nauplii were fed to the *C. magister* zoeae at a density of three per millilitre, and the culture water was replenished twice each week. The experiment was terminated after 72 d when over 80% of the surviving zoeae had metamorphosed to the megalops stage.

Results

Variable Duration of Antibiotic Treatment

The average survival of crabs to the megalops stage for each antibiotic treatment was A) 9% no treatment; B) 44% antibiotics every water change; C) 56% every second water change; D) 56% every fourth water change; E) 46% first 2 water changes only; F) 30% first 5 water changes only; and G) 23% first 10 water changes only (Fig. 1). The untreated controls showed a daily mortality rate of 2.5% in the first 20 d, while the poorest antibiotic-treated condition (F) showed only a 0.7% decrease in survival. Mortalities also occurred in nearly all cases about 55–60 d after hatching.

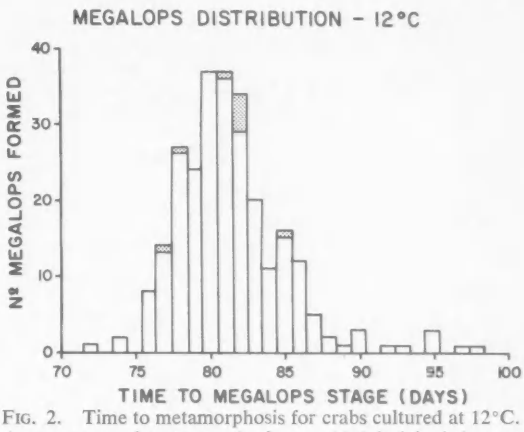

FIG. 2. Time to metamorphosis for crabs cultured at 12°C. Average age of metamorphosis was 81.4 d. Stippled areas represent control (untreated) crabs that metamorphosed at an average 81.2 d.

A distribution chart combining all the conditions (Fig. 2) shows the shortest time to metamorphosis was 72 d while the average was calculated to be 81.4 d. A few crabs remained in the fifth zoeal stage after 100 d. The average age to metamorphosis of untreated control crabs was 81.2 d.

The wet and dry weights of the cultured crabs after 79, 107, and 112 d, and wild crabs at a similar developmental stage are represented in Table 1. The dry weight of 107-d untreated cultured animals and the antibiotic-treated cultured animals was only 20.2 and 38.5%, respectively, of the dry weight of wild animals. By 112 d the antibiotic-treated cultured crabs weighed 56.8% of the dry weight of the wild crabs. The wet/dry ratio for captured crabs was 3:1, while antibiotic-treated animals were 4:1 and untreated cultured crabs 5:1 after 107 d. The antibiotic treated crabs also showed a 4:1 ratio after 112 d.

Molting in this experiment was nearly synchronous throughout all conditions. Molting periods occurred near the ages of 14, 26, 38, and 50 d.

Comparison of Various Types and Concentrations of Antibiotics

The results of the initial screening for effective antibiotic concentrations against microorganisms from a crab culturing system are shown in Fig. 3. The greatest inhibition of microbial growth (turbidity) occurred with 10 and 50 mg/L chloramphenicol, although a 1 mg/L

TABLE 1. Wet and dry weights (mg) ± SD of wild crabs and cultured crabs at three different stages. The ages (in parentheses) are known only for the cultured crabs, while the approximate stages of the wild crabs were determined by comparison with nearly 100 apparent siblings captured at the same time. The sample size was $n = 10$ except where noted. The average dry weight for the 107-d untreated control crabs was obtained by weighing the four animals together.

Approximate stage (age)	Wild	Cultured	
		Untreated control	Antibiotic treated
1–2-d megalops (79 d)	—	—	dry 2.36 ± .91
late megalops (107 d)	wet 48.6 ± 5.1 dry 15.9 ± 2.1 ($n = 20$)	16.5 ± 4.7 3.2 ($n = 4$)	24.6 ± 3.1 6.1 ± 1.6
5-d instars (112 d)	dry 22.8 ± 6.8	—	wet 51.9 ± 9.9 dry 13.0 ± 3.0

concentration was also quite effective. Neomycin concentrations of 50 and 100 mg/L showed nearly identical activities, both notably more potent than the 1 and 10 mg/L concentrations. Kanamycin showed increased effectiveness with increased concentration from 1 to 100 mg/L, with the greatest increase in effectiveness from 50 to 100 mg/L.

The final antibiotic screening (Fig. 4) showed treatment with chloramphenicol to cause a reduction in optical density with increased concentration from 1.0 to 7.5 mg/L. Streptomycin and penicillin (100 mg/L each) were not particularly effective singly or in combination, although there was a reduction over the first 3 d relative to the untreated control. Chloramphenicol (1 mg/L) and neomycin (50 mg/L) were more effective in combination than they were singly. Similarly, the combined effects of 1 mg/L chloramphenicol and 100 mg/L kanamycin were greater than each used separately. Kanamycin (100 mg/L) and neomycin (50 mg/L) were no more effective in combination than they were singly.

The effects of various concentrations and combinations of these antibiotics on crab survival are presented in Fig. 5. Concentrations of 1, 5, and 10 mg/L chloramphenicol maintained the highest survivals with the

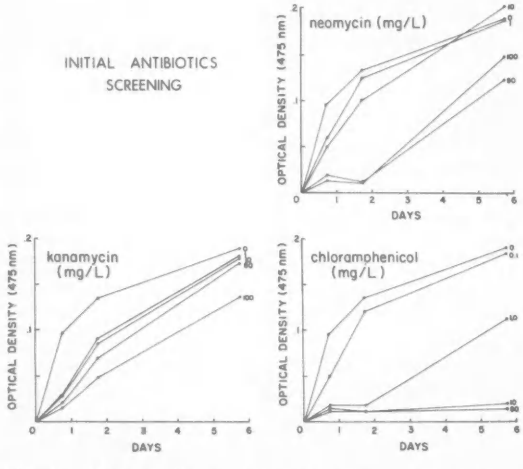

FIG. 3. Growth of bacteria from a crab culture system incubated with various concentrations of the antibiotics neomycin, kanamycin, and chloramphenicol.

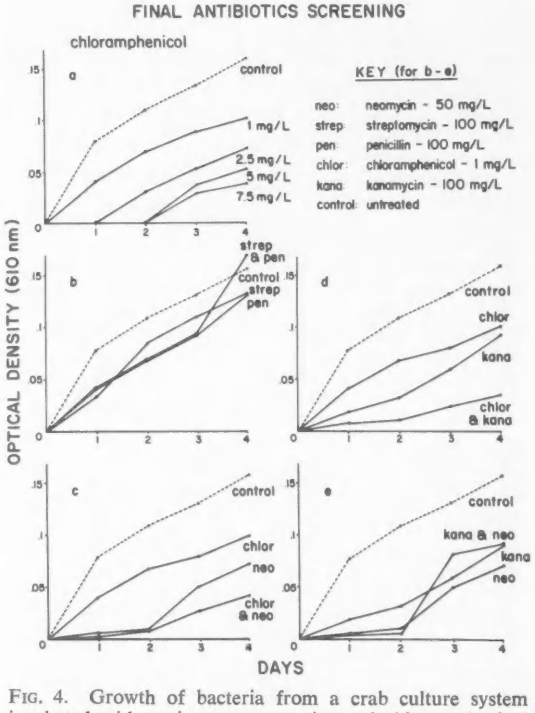

FIG. 4. Growth of bacteria from a crab culture system incubated with various concentrations of chloramphenicol (a) or combinations of streptomycin and penicillin (b), neomycin and chloramphenicol (c), kanamycin and chloramphenicol (d), and kanamycin and neomycin (e). Antibiotic synergism is evident in (c) and (d) where chloramphenicol is combined with neomycin or kanamycin.

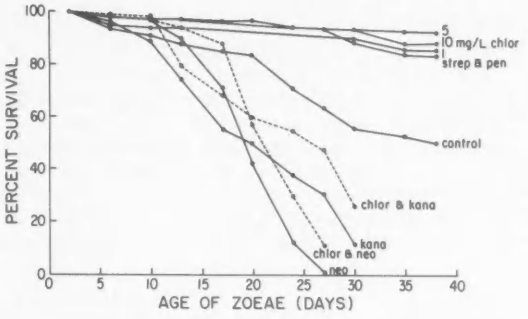

FIG. 5. Average zoeal survival from three replicates of various twice weekly antibiotic treatments. Survival less than the untreated controls was exhibited by treatments that included kanamycin or neomycin. Chlor — chloramphenicol; Strep — streptomycin; Pen — penicillin; Kana — kanamycin; Neo — neomycin.

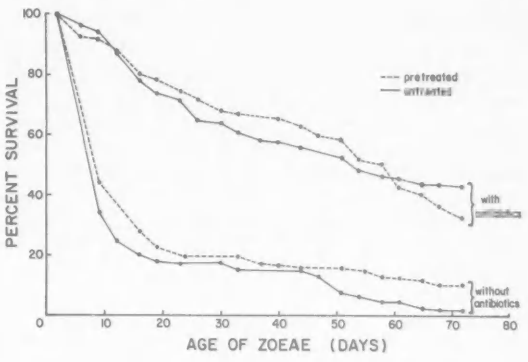

FIG. 6. Average zoeal survival from three replicates of 50 animals each fed pretreated or untreated *A. salina* nauplii and cultured with or without the addition of antibiotics.

TABLE 2. Bacterial counts from seawater in which treated *A. salina* nauplii were held. *Artemia salina* nauplii were pretreated with antibiotics (100 mg/L streptomycin plus 100 mg/L penicillin) or with 10 mg/L malachite green.

	Posttreatment samples		
	24 h	48 h	72 h
Antibiotics (0 h)	8.2×10^3	2.9×10^5	4.3×10^5
(2 h)	9.6×10^2	8.3×10^4	1.0×10^5
(4 h)	1.8×10^3	2.1×10^5	1.2×10^6
(6 h)	4.7×10^2	2.1×10^4	4.0×10^5
Control (no nauplii)	2.3×10^1	4.1×10^2	4.2×10^3
Malachite green (0 h)	3.5×10^3	8.3×10^4	3.3×10^5
(2 h)	1.3×10^3	1.7×10^5	1.8×10^6
(4 h)	1.0×10^3	5.6×10^4	3.0×10^5
(6 h)	1.4×10^3	3.0×10^4	8.0×10^5

5 mg/L treatment slightly higher (91% survival) after 38 d. Treatment with 100 mg/L each of streptomycin and penicillin held zoeal survival at 84%. The untreated control zoeae had 51% survival in this period. Kanamycin (100 mg/L) and neomycin (50 mg/L) showed a decrease in zoeal survival with and without the addition of 1 mg/L chloramphenicol to less than 15% by 30 d. Microscopic examination of representative zoea from each test showed antibiotic-treated crabs to appear devoid of microbial fouling, while control animals became highly infested with several forms of microorganisms including bacteria, algae, and protozoans.

THERAPEUTIC PRETREATMENT OF LIVE FOOD

The treatment of *A. salina* nauplii with antibiotics or malachite green resulted in the bacterial counts shown in Table 2. Nauplii treated with antibiotics for 2 h showed bacterial populations in the culture water to be about an order of magnitude less than the untreated

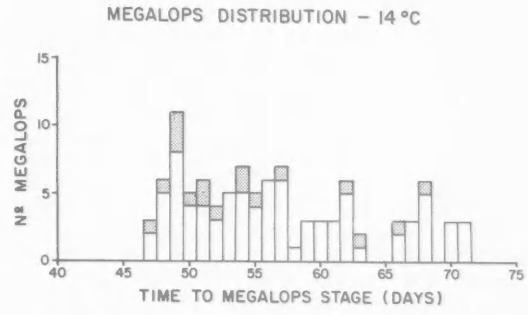

FIG. 7. Ages at metamorphosis for crabs cultured at 14°C. Average age at metamorphosis was 57.2 d, although 18% of the original zoeae had not metamorphosed before termination of the experiment (72 d). Stippled areas designate untreated control animals.

control after 24 h. No further inhibition was exhibited by the extended exposures of 4 and 6 h. The bacterial populations of the treated nauplii had increased relative to the control by 48 h and were nearly identical after 72 h. The treatment of nauplii with malachite green (10 mg/L) had very little influence on the bacterial populations regardless of the exposure period.

Crab zoeae fed *A. salina* nauplii that had been pretreated with antibiotics (10 mg/L chloramphenicol) showed a slightly higher survival throughout the majority of the study. Survival data, averaged from three replications, are presented in Fig. 6. The distribution of ages at metamorphosis are presented in Fig. 7. Over 82% of the crabs (held at 14°C) had metamorphosed by 72 d when the experiment was terminated. At this time, the average age at metamorphosis was 57.2 d and the earliest megalops was formed at 47 d.

Discussion

Antibiotic treatment of *C. magister* zoeae has been shown to be most effective when administered 1–2 times

per week throughout the culture period (Fig. 1). Rate of zoeal development was not slowed by antibiotics since zoeae from both treated and untreated conditions metamorphosed at the same age (Fig. 2 and 7). The sizes of the zoeae were not decreased by antibiotics as previously suspected (Fisher and Nelson 1977), even though all of the cultured zoeae were undersized when compared with wild crabs (Table 1).

Untreated crabs represented in Fig. 1 had a daily mortality rate of 2.5% over the first 20 d, while the antibiotic-treated zoeae showed, at worst, a 0.7% rate, indicating that the beneficial effects of antibiotics occurred during this early period. This suggestion is further supported by the relative success of zoeae treated on only the first two water changes (Fig. 1C). Antibiotic treatment was not beneficial at 55–60 d when nearly all conditions showed a decline in survival. This later decline does not appear to be related to bacterial involvement.

Comparison of the dry weights between wild crabs and crabs cultured with or without antibiotics (Table 1) shows a dramatic stunting of the cultivated crabs as previously reported by Hartman (1977). The crabs cultured with antibiotics were nearly twice the dry weight of the untreated cultured crabs and had a wet dry weight ratio more closely resembling wild crabs. The increase in dry weight for antibiotic cultured crabs relative to the wild crabs between late megalops and 5-d instars may be an artifact since the wild crabs were maintained in culture conditions over this 1-wk period.

Concentrations of 1, 5, and 10 mg/L chloramphenicol were able to sustain zoeal survival equal to the success of penicillin and streptomycin (Fig. 5) and were able to control bacteria with greater success (Fig. 4). The addition of 50 mg/L neomycin or 100 mg/L kanamycin caused zoeal mortalities greater than the untreated controls (Fig. 5), suggesting a toxic effect. Lower concentrations of these two antibiotics and/or shorter treatment periods will be necessary to determine any beneficial applications to crab cultivation.

Antibiotic treatment of the live food, *A. salina* nauplii, decreased the bacterial population by an order of magnitude for the first 24 h (Table 2), but yielded only a slight increase in crab zoeae survival when they were fed the pretreated *A. salina* nauplii (Fig. 6). This does not disqualify the possibility that the live food is partially responsible for bacteria involved in zoeal mortality, since the pretreatment may not have sufficiently controlled the bacteria to allow a significant change in zoeal survival.

It is not entirely acceptable to compare the results between experiments in this study since the zoeae came from different hatches, and different hatches may vary significantly. However, it is possible to observe that the water temperature affected the age of metamorphosis as previously shown by Reed (1969). Zoeae represented in Fig. 2 were held at 12°C while those of Fig. 7 were maintained at 14°C. The average age at metamorphosis in the 12°C experiment was 81.4 d and 14°C zoeae reached megalops after an average 57.2 d. Since 18% of the zoeae in the latter experiment had not metamorphosed when the experiment was terminated (72 d), it is likely that 57.2 d is, however, a low estimate of the average age of metamorphosis. It is also apparent by comparing Fig. 2 and 7 that there was a wider range of ages at metamorphosis in the warmer temperature, indicating less molting synchrony.

We observed that antibiotic treatment of the crab zoeae restricted the growth of algae and protozoans as well as bacteria. Rather than acting directly on the nonbacterial epibionts, it is more likely that the elimination of the bacteria which may establish a mat or mucus on the zoeal surface, lessened the capacity of other forms to settle. The restrictions of nonbacterial forms with antibiotics contradicts earlier *C. magister* egg studies (Fisher 1976), but the concentration of streptomycin and penicillin on the eggs was only 1 mg/L which may not have eliminated the bacteria sufficiently to restrict nonbacterial growth.

Fisher (1977) noted that the necessity for antibiotics in Dungeness crab cultivation has been limited to those areas in central California that have also shown a decline in the natural crab fishery, possibly due to increased nutrient in the San Francisco Bay effluent (Fisher and Wickham 1976). Increased nutrient loads on the eggs of *C. magister* have been shown to increase microbial epibiotic infestation and decrease egg survival (Fisher 1976). Evidently parameters affecting bacterial populations will also affect the survival of the early life stages (egg and zoea) of the Dungeness crab, both in nature and in culture.

Acknowledgments

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Parasitic Mastitis in the Atlantic White-Sided Dolphin, *Lagenorhynchus acutus*, as a Probable Factor in Herd Productivity

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In a survey of 30 female Atlantic white-sided dolphins, *Lagenorhynchus acutus*, taken from a mass stranded herd of approximately 150 animals, 47% were found to be infected with *Crassicauda grampicola* (Nematoda: Spiruridae) in the mammary glands. Associated lesions included verminous cysts, fibrosis, necrosis and inflammation, and destruction or replacement of normal parenchyma by nonsecretory tissue. The high incidence and severity of these lesions are viewed as potentially compromising the reproductive success of the herd. The life cycle of *C. grampicola* is discussed in relation to its presence in the mammary gland.

Key words: cetacean, Atlantic white-sided dolphin, pathology, nematodes, mastitis, population, nutrition

GERACI, J. R., M. D. DAILEY, AND D. J. ST. AUBIN. 1978. Parasitic mastitis in the Atlantic white-sided dolphin, *Lagenorhynchus acutus*, as a probable factor in herd productivity. J. Fish. Res. Board Can. 35: 1350–1355.

Nous avons examiné 30 dauphins francs-blancs femelles de l'Atlantique, *Lagenorhynchus acutus*, membres d'un troupeau d'environ 150 qui s'était échoué en masse. Nous avons constaté que 47% étaient infestés de *Crassicauda grampicola* (Nematoda: Spiruridae) dans les glandes mammaires. Les lésions accompagnant cette infestation sont les suivantes : kystes vermineux, fibrose, nécrose et inflammation, et destruction ou remplacement du parenchyme normal par un tissu non secréteur. Nous sommes d'avis que la forte incidence et la sévérité de ces lésions peuvent compromettre la reproduction du troupeau. Nous examinons le cycle biologique de *C. grampicola* en relation avec sa présence dans la glande mammaire.

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PARASITES have been identified from virtually every organ and tissue of cetaceans (Delyamure 1955; Dailey and Brownell 1972). Few have been shown to cause more than local disturbance. Notable exceptions are the many species of lung nematodes that are associated with pneumonia, especially in captive cetaceans (Woodward et al. 1969), and the cranial sinus trematodes which can invade brain tissue causing debilitating cerebral necrosis and hemorrhage (Ridgway and Dailey 1972). Some functional disturbances have also been associated with the spirurid nematode *Crassicauda crassicauda* in the ureters and kidney of baleen and toothed whales (Baylis 1961; Cockrill 1960).

The present report deals with a related nematode, *C. grampicola*, in the mammary glands of the Atlantic white-sided dolphin, *Lagenorhynchus acutus*. Its presence is described in relation to functional impairment and possible consequences on reproductive success of the host.

Materials and Methods

On September 6, 1974, approximately 150 *L. acutus* stranded at Lingley Cove, Edmunds, Maine. Forty-three dolphin carcasses were collected and stored in a commercial

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freezer for 6 mo prior to detailed postmortem examination. Teeth were collected from all specimens for age determination (Sergeant 1959). Mammary tissue from 30 female dolphins was examined grossly and samples from 25 were fixed in 10% buffered formalin for histopathology. Sections were cut at 6 μm and stained with hematoxylin and eosin, Masson's trichrome, and periodic acid-Schiff. Parasites were placed in alcohol–formalin–acetic acid fixative, cleared in glycerin–alcohol (70% ETOH), and mounted in glycerin jelly.

Milk samples from lactating females were preserved frozen for analysis of composition and microscopic examination. Nematode ova from the thawed milk samples were isolated and preserved in 70% ethanol containing 5% glycerin.

Results

The paired mammary glands of *L. acutus* lie along the ventral abdominal wall, on either side of the genital slit. Measurement of 37 glands showed a range of 28–50 cm (mean ± SD, 42 ± 4) long, 4–12 cm (8 ± 2) wide, and 0.8–3.5 cm (2.2 ± 0.7) thick. The mean weight determined from 13 glands was 630 ± 240 g. Each firm, compact gland has a number of lactiferous ducts which join to form a large central lactiferous canal running longitudinally near the dorsal surface of the gland adjacent to the abdominal musculature (Arvy 1973–74). The canal widens caudally into a sinus before terminating at the narrow mammary cone (Fig. 1).

In 14 of the 30 females examined, the central lactiferous canal contained large nematodes lying either free within the duct or firmly embedded in one of the smaller lactiferous ducts (Fig. 2). In one case, the head end of the nematode was traced through the mammary gland to a firm nodule containing a verminous coil within the abdominal muscle overlying the gland. Similar nodules were found on the surface of the glands of six dolphins. They were thin walled and contained cream-colored gelatinous material with evidence of dissolving nematodes (Fig. 3).

Nearly all of the glands in which the nematodes were found appeared to have some degree of inflammation. Affected glands contained thick straw-to-reddish colored milk. The glands had a mottled appearance, resulting from small, up to 1 cm diameter, pale depressions scattered over much of the surface and extending well into the gland parenchyma. On cut section, the lesions had a green cast; they were well demarcated and wedge-shaped, with radiation outward to the surface of the gland (Fig. 4). Histologically, these lesions represented clearly defined areas of fibrosis. The abundant fibrous tissue appeared to result from involution of lobules, with subsequent condensation of intralobular connective tissue, as well as new connective tissue formation.

The alveolar and ductal epithelium of the affected areas showed varying degrees of hypertrophy, hyperplasia, and squamous metaplasia (Fig. 5A). The ducts were obviously plugged in some cases, resulting in dilated tortuous fluid-filled cysts. Scattered throughout the affected glands were small, dark-green granules which imparted a gritty texture. Microscopically, these appeared as mineralized debris in the ducts, associated with fragments of calcified nematodes. In addition, ova were observed in various stages of degeneration, including calcification (Fig. 5A). One such ovum was observed at the center of a laminated eosinophilic concretion, possibly the result of inspissated milk or other secretory material (Fig. 5B).

TABLE 1. Summary of gross and microscopic findings in the mammary glands of *L. acutus* females.

Gross findings (30 examined)	No. of dolphins affected
Nematodes in ducts or encysted	14
Cysts	6
Ova in milk (12 specimens examined)	2
Calculi, and calcified nematodes	6
Demarcated discolorations, wedge-shaped lesions	7
Firm gland, areas of fibrosis	6
Mastitis	2
Abscesses	1
Plaques in duct	1
No gross lesions	7
Microscopic findings (25 examined)	
Nematode ova	17
Nematodes, intact or degenerating	4
Mineralization of debris	12
Concretions	9
Connective tissue proliferation	13
"Infarcted" lobules	6
Epithelial hyperplasia and/or hypertrophy	9
Mastitis	15
Focal	13
Diffuse	5
Mild	12
Severe	3

Generally, the mammary lesions were associated with a mild leukocytic reaction consisting of focal mononuclear cell infiltration, especially in the thickened interlobular septa. The three most severely affected animals had a more generalized mononuclear cell infiltration and marked fibroplasia involving much of the gland parenchyma. The youngest infected dolphin was 2 yr old; no correlation between age and severity could be determined, since the sample was biased toward adult dolphins. Gross and microscopic findings are summarized in Table 1.

Examination of the milk in seven dolphins in which apparently viable nematodes were found revealed the presence of nematode ova in two cases. The ova were round to slightly eliptical and possessed clearly visible capsules (Fig. 5C). Fifty ova (in tissue sections) from nine cases measured 26.9–39.9 μm (mean ± SD, 28.7 ± 4.9 μm) long by 18.6–27.9 μm (25.1 ± 2.9 μm) wide. Ova were not observed in the milk of three dolphins with calcified or dissolving nematodes in the mammary glands.

Description of Nematode

Crassicauda grampicola was originally described by Johnston and Mawson (1941) from a Risso's dolphin (*Grampus griseus*) taken in Australian waters. Skrjabin (1969) lists 13 species in the genus *Crassicauda*. In all cases, except possibly *C. boopis* Baylis, 1920, no entire worms have been recovered. Extraction of intact worms is nearly impossible due to the tissue involvement associated with the parasite. The specimens recovered during this study were badly fragmented with the longest portion (anterior end through the glandular esophagus) measuring 25 cm long. Measurements and numbers of papillae conform to those taxonomic criteria given for *C. grampicola*.

Discussion

Lagenorhynchus acutus represents a new host record for *C. grampicola* and is the first species in which this parasite has been recorded from the mammary glands. However, the record of parasites in the cetacean mammary glands dates back to the findings of Howell (1927) who described "a single long vermiform parasite" within the mammary duct, and "a number of smaller parasitic worms" encysted in the glandular tissue from a finless porpoise, *Neophocoena phocoenoides*. The parasites were not identified, nor were any associated lesions reported. *Crassicauda crassicauda*, recovered from the mammary gland of the bottlenosed dolphin, *Tursiops tursio*, was the first nematode identified from this organ in a cetacean (Joyeux and Baer, 1931). Cockrill (1960) described a case of acute mastitis in a blue whale, *Balaenoptera musculus*, the diagnosis of which was based on gland enlargement, firm consistency, and blood-tinged milk. No nematodes were reported.

Ridgway (1966) found numerous worms in the ducts of the mammary glands of a Dall's porpoise, *Phocoenoides dalli*. These worms, which were identified as *Placentonema* sp., were also found in several subcutaneous nodules on the "posterior half" of the body. The nodules contained only females; the mammary ducts contained both sexes. This case was redescribed together with another *Phocoenoides dalli* in which similar findings were observed and were associated with lobular hyperplasia (Johnston and Ridgway 1969). In contrast with our study, no significant fibrous or cellular infiltration was observed.

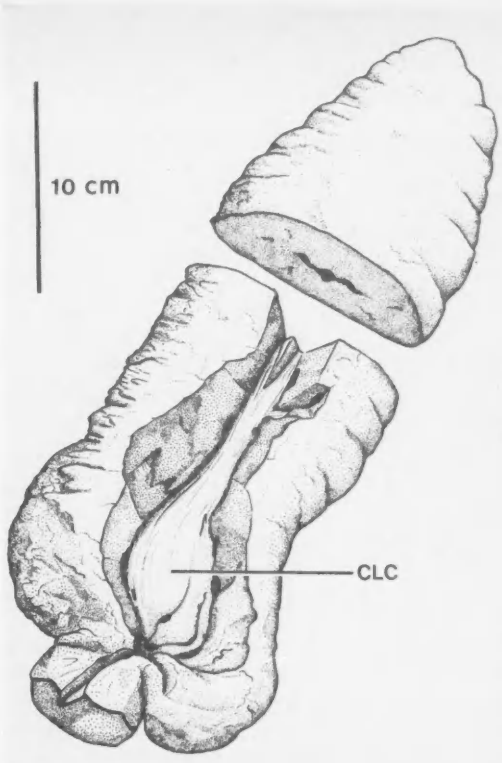
Placentonema sp. is a nematode inhabiting the placenta of the sperm whale, *Physeter catodon* (Delyamure 1955), whereas *Crassicauda* spp. are found ubiquitously in the urinary passages of cetaceans (Arvy 1973–74). *Crassicauda grampicola* in the present study behave in a manner similar to crassicaudids in the urinary system with respect to their location within ducts and tubes and the formation of verminous nodules (Baylis 1922). Worms of the genus *Placentonema* are distinctly different from *Crassicauda* in size (the female reaches 9 m long by 2.5 cm wide) and location (transparent placental lining). In addition, the body cuticle is transparent, whereas that of the genus *Crassicauda* is opaque. In light of our present knowledge, and without specimens for confirmation, it is probable that the *Phocoenoides dalli* parasites reported by Ridgway (1966) and Johnston and Ridgway (1969) were a species of *Crassicauda*. This is further supported by a study just completed (Dailey and Walker unpublished data) in which three or four *P. dalli* examined in southern California were found parasitized with *Crassicauda* sp. The mammary glands were among the tissues infected.

Crassicauda sp. in this study were also found in the fascia underlying blubber along the lateral thoracic and abdominal body wall and rarely in verminous nodules within muscle (Geraci et al. 1976). Species identification was not possible due to the difficulty in extracting entire worms, but eggs measured in sections of muscle cysts were identical in size (mean of 20 eggs, 29.6×23.6 μm) to those of *C. grampicola*. Dailey and Perrin (1973) reported a similar finding in a spinner porpoise, *Stenella longirostris*. The relationship of *Crassicauda* sp. in the muscle to *C. grampicola* in the mammary gland remains unknown. Crassicaudids are found in various cetacean organs and tissues (Baylis 1922; Arvy 1973–74; Robineau 1975) and there is some indication that they may all be the same parasite with slight morphological variations (M. D. Dailey, unpublished data).

Brief mention should be made of the probable life cycle of *C. grampicola*. The taxonomy of crassicaudids is uncertain. It is generally listed in the order Spiruridae which includes a potpourri of nematodes, most of which are found in the stomachs of the hosts. This group utilizes an intermediate host in its development, usually an arthropod (Schmidt and Roberts 1977). This feature of the life cycle would suggest that dolphin calves probably do not become infected by direct egg transmission from the milk. This is supported by the finding that the youngest infected dolphin was 2 yr old. More likely, the calf serves as the vehicle to disseminate

FIGURES 1–4

FIG. 1. Caudoventral view of one *L. acutus* mammary gland, with exposed central lactiferous canal (CLC). FIG. 2. Parasitic nematodes (P) *C. grampicola* within the central lactiferous canal. Anterior ends of the nematodes course through smaller tributary ducts and are firmly embedded in parenchyma. The largest segment retrieved measured 25 cm. FIG. 3. Thin-walled cyst on the surface of the mammary gland adjacent to the body wall. It contained cream-colored gelatinous material and nematode fragments. FIG. 4. Cross section through typically affected mammary gland. Surface has a mottled appearance resulting from areas of fibrosis (outlined with dots). Dark stippling is due to dark green granules which represent calcified parasites and ova.



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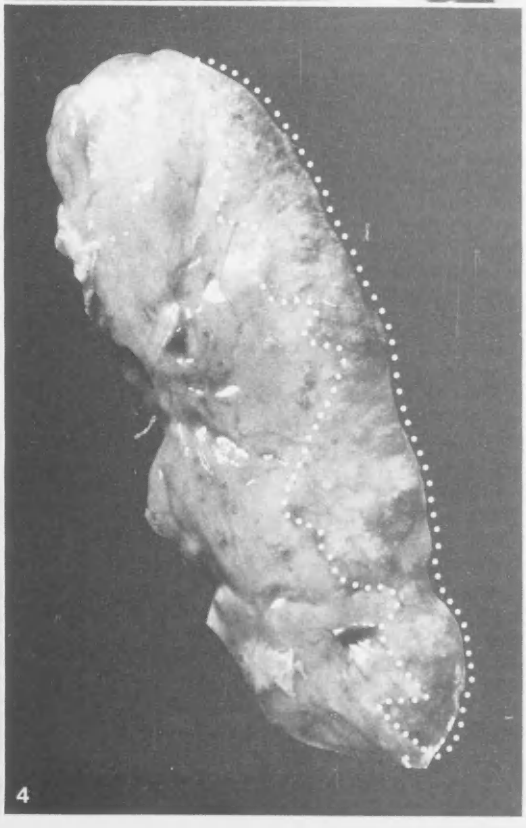

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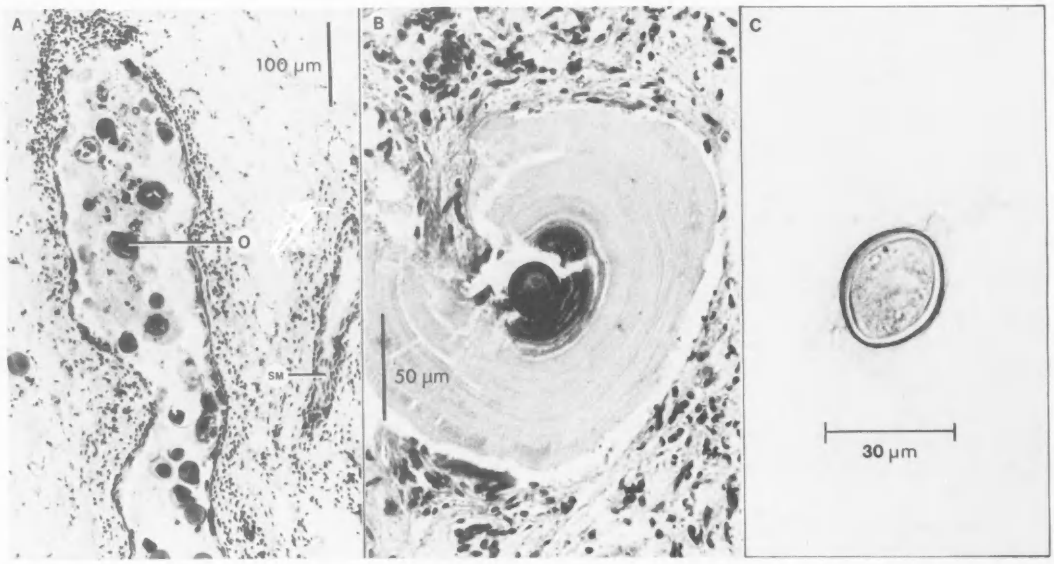

FIG. 5. A, Photomicrograph of a small mammary duct containing nematode ova (O) and cellular debris. The lining cuboidal epithelium has changed to a nonsecretory squamous type (squamous metaplasia — SM). B, Calcified nematode ovum surrounded by laminated, eosinophilic inspissated material. These structures imparted a gritty texture to the gland. C, Ovum of *C. grampicola* taken from milk of an infected dolphin.

the otherwise imprisoned eggs into the environment by passing the ova in its feces. Transmission and maturation through intermediate hosts would then occur after ingestion of the ova along with planktonic food. Once in the mammary glands, the adult nematodes could well be dormant during the time of glandular inactivity when egg transmission is impossible, then be stimulated into activity by hormonal mediation of the host. This mechanism has been demonstrated in the life cycle of the rabbit flea, *Spilopsyllus cuhiculi*, whose ovarian maturation occurs only on pregnant rabbits under the influence of host corticosteroids (Rothschild 1965). This same physiological triggering mechanism has been observed in the monogenetic trematode of frogs, *Polystoma integerrimum* (Smyth 1966) and represents a possibility in crassicaudid parasite transmission. Resolution of this problem requires examination of calf feces for *Crassicauda* eggs and close examination of phyletic relationships between this nematode and others for which the life cycles are known.

The high prevalence and severity of mammary lesions within the *L. acutus* herd must be viewed with regard to the possible influence on reproductive success. The most dramatic lesions were found in animals ranging from 8 to 15 yr old. In this group, the marked fibroplasia, necrosis and inflammation, and squamous metaplasia of alveolar and ductal epithelium represent a replacement of functional parenchyma by nonsecretory tissue in which milk cannot be produced. Total milk production would be affected only if the degree of damage exceeded the functional reserve of the gland. In many cases, there appeared to be sufficient damage to do so, despite the obvious compensatory (hyperplasia) attemps of the glands to regenerate secretory cells.

The firm fibrous tissue replacement evident in some of the glands might further bias milk output which depends on forceable abdominal muscular compression of a normally pliable gland in order to extrude milk to the suckling calf. In addition to the possible lowering of milk production and output, milk quality might well be reduced by the presence in the milk of parasite and tissue debris, ova, and inflammatory cells. We suggest that these factors, in the extreme, may result in nutrient insuffiicency leading to malnutrition and reduced growth rate, which in turn could bias calf survival and thereby influence herd productivity.

The exact significance of the mammary gland lesions is still speculative and awaits the results of studies on gland morphology, physiology, and biochemistry of milk secretion in cetaceans. Ultimately, studies must be carried out on wild-caught specimens so as to compare progressive changes in these parameters in variously affected dolphins.

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Helminths of the Alimentary Tract from a Stranded Herd of the Atlantic White-Sided Dolphin, *Lagenorhynchus acutus*

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BEVERLEY-BURTON, M. 1978. Helminths of the alimentary tract from a stranded herd of the Atlantic white-sided dolphin, *Lagenorhynchus acutus*. J. Fish. Res. Board Can. 35: 1356–1359.

Six species of helminths were collected from the gut of 37 *Lagenorhynchus acutus*. The following are new host–parasite records: *Pholeter gastrophilus*, *Oschmarinella laevicaecum*, *Tetrabothrius forsteri*, *Anisakis* sp., and *Bolbosoma* sp. *Stenurus globicephalae* was also found. Prevalence for *O. laevicaecum* was 49% and for *T. forsteri* 78%.

Key words: Atlantic dolphins, digenea trematodes, cestodes, nematodes, acanthocephala, biological tags

BEVERLEY-BURTON, M. 1978. Helminths of the alimentary tract from a stranded herd of the Atlantic white-sided dolphin, *Lagenorhynchus acutus*. J. Fish. Res. Board Can. 35: 1356–1359.

Six espèces d'helminthes ont été recueillies dans le tractus digestif de 37 *Lagenorhynchus acutus*. Les espèces suivantes sont des signalements hôte–parasite nouveaux : *Pholeter gastrophilus*, *Oschmarinella laevicaecum*, *Tetrabothrius forsteri*, *Anisakis* sp. et *Bolbosoma* sp. On a également trouvé *Stenurus globicephalae*. La prévalence est de 49% pour *O. laevicaecum* et de 78% pour *T. forsteri*.

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INFORMATION on the helminthic parasites of the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) is restricted to isolated necropsy reports which have subsequently been incorporated into host–parasite listings (Delyamure 1955; Dailey and Brownell 1972).

A growing awareness of the role of certain parasite species as biological tags (Margolis 1963; Kabata 1963; Pippy 1969; Gibson 1972; Beverley-Burton and Pippy 1977, 1978) for host stock identification and feeding behavior studies accentuates the need for work on carefully gathered collections. The present study, describing the first comprehensive collection of gut helminths to be taken from a sizable group (37) of white-sided dolphins and including prevalence and intensity data, provides basic preliminary data on the parasitic fauna.

Materials and methods — On September 6, 1974, approximately 150 *L. acutus* were stranded at Lingley Cove, Edmunds, Maine, USA. Many of the dolphins survived and returned to sea on the high tide or were rescued (Geraci et al. 1975). All of the accessible carcasses (43) were trucked to a commercial freezer and stored for 6 mo. Subsequently, the alimentary tracts from 37 of these animals (29 females and 8 males) were made available for helminthological examination. The whole gut was removed from thawed carcasses and measured. The intestine was cut into 2-m sections and opened so that the gut wall could be scrutinized under a dissecting microscope. Worms were removed, fixed, and stored in cold alcohol-formol-acetic.

Whole mounts of digenes and cestodes were prepared from material stained with Erlich's hematoxylin or acetocarmine; sections of paraffin-embedded digenes were stained with hematoxylin and eosin. Nematodes were cleared in glycerin in alcohol or lactophenol; en face preparations were made of representative specimens. Acanthocephalans were cleared in lactophenol.

Results and discussion — The parasites found in the gut consisted of two digenes, one cestode, two nematodes, and one acanthocephalan.

Stenurus globicephalae has been recorded previously from *L. acutus* by Arnold and Gaskin (1975). Baer (1932) listed *Strobilocephalus triangularis* as a parasite of *L. acutus* and *Hyperoodon rostratus* (syn. of *ampullatus*), but later Baer (1954) noted that both records were erroneous. Delyamure (1955) and Dailey and Brownell (1972) were, apparently, unaware of Baer's

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(1954) corrections. Thus, the following are new records: *Pholeter gastrophilus*, *Oschmarinella laevicaecum*, *Tetrabothrius forsteri*, *Anisakis* sp., and *Bolbosoma* sp. *Stenurus globicephalae* was also found.

Description of helminths and comments

DIGENEA

Pholeter gastrophilus (Kossack, 1910): Troglotrematidae

A single specimen was found which measured 3.7 mm long by 1.57 mm maximum width; eggs 22 μm by 12 μm. Morphologically this specimen is similar to the description included in Delyamure (1955).

Pholeter gastrophilus normally occurs in cysts within the pyloric mucosa (Dawes 1946; Delyamure 1955). The stomach mucosa was carefully examined but there was no gross evidence of cysts although histologically digeneans were found in two of seven stomachs sectioned. The specimen in the present collection was found in a cyst in the duodenum of a male dolphin weighing 197.2 kg and measuring 254 cm long.

Oschmarinella laevicaecum (Yamaguti, 1942): Campulidae

Ten gravid specimens measured 6.0–13.5 mm long by 0.99–1.50 mm in maximum width. Oral sucker 0.48–0.61 mm diam; prepharynx 47–226 μm long; pharynx 0.34–0.43 mm long by 0.20–0.30 mm; anterior ceca extended to posterior region of oral sucker. Ventral sucker 0.40–0.57 mm diam. Testes situated in middle third of body measuring 0.85–2.03 mm long by 0.41–0.74 mm, margins indented or lobate; cirrus pouch 0.87 mm long, cirrus apparently unarmed; ovary 0.22 × 0.29–0.37 mm diam; metraterm without spines. Eggs 87–96 μm long by 37–43 μm wide. Ani present.

The "liver flukes" were taken from the bile ducts and hepatopancreatic ducts. Although obviously of the family Campulidae, assignment of the material to a genus presented difficulties. Skrjabin (1948), Delyamure (1955), and Yamaguti (1971) have included keys for the separation of campulid genera, but there are inconsistencies indicating the need for a detailed taxonomic study which is outside the scope of the present work. The worms most closely resemble *laevicaecum* described by Yamaguti (1942) which is, at present, included in the genus *Oschmarinella*.

The prevalence and intensity of infections with this trematode are noted below.

CESTODA

Tetrabothrius forsteri (Krefft, 1871): Tetrabothriidae

The cestodes were generally poorly fixed but 25 intact specimens were selected for examination. Worms measuring 45 mm long carried mature proglottides; those measuring 110 mm carried gravid proglottides. Mature proglottides averaged 1.6 mm wide, although one contracted length of strobila had segments 3.0 mm wide. The shape and size of the scolex varied with the state of preservation. Unmounted scoleces 1.1–1.4 mm wide carried bothridia similar to those depicted by Baer (1954) for *T. forsteri*. From 19 to 25 testes were present in each segment; cirrus sac 74–93 μm long by up to 50 μm wide; cirrus apparently unarmed. Vitellarium 90 μm diam by 60 μm; eggs 44–48 μm diam; oncospheres 34–42 μm by 23–31 μm.

The worms were readily recognized as *Tetrabothrius* sp. but there has been considerable confusion concerning the identification and synonymy of the species of this genus. However, following the synonomy used by Baer (1954), the present material most closely resembles *T. forsteri*.

The prevalence and intensity of infections with this cestode are noted below.

NEMATODA

Anisakis sp.: Anisakidae

A single female was found in the stomach of a 12-yr-old female dolphin.

Stenurus globicephalae Baylis and Daubney, 1925: Pseudaliidae

Three female specimens were found in the stomach of a female dolphin weighing 171.19 kg. *Stenurus globicephalae* normally occurs in the lungs, blowhole, and cranial sinuses of dolphins (Delyamure 1955; Arnold and Gaskin 1975). These "lung worms" had probably been passed up the trachea, were swallowed, and thus were transitory in the stomach.

ACANTHOCEPHALA

Bolbosoma sp.: Polymorphidae

A single male specimen (20 mm long) was found in a female dolphin weighing 137.7 kg. It was not possible to count the number of longitudinal rows of hooks, hence a specific identification has not been made.

Prevalence and intensity of O. LAEVICAECUM, T. FORSTERI, *and* BOLBOSOMA *sp. infections*

Oschmarinella laevicaecum was found in the liver, bile ducts, and hepatopancreatic ducts of 18 dolphins (14 females and 4 males) which represents a prevalence of 48.7%. Up to 200 campulids were found in individual dolphins. Numbers found are not maxima as no attempt was made to collect all specimens present.

Tetrabothrius forsteri was found in 29 dolphins (24 females and 5 males) which represents a prevalence of 78.4%. Most worms (>95%) were found in the first 8 m of the intestine (Fig. 1). Intensity, established from the numbers of scolexces, varied from 2 to 110. Intensity was related to age, using the dentinal growth layers technique of Sergeant (1959), and body length

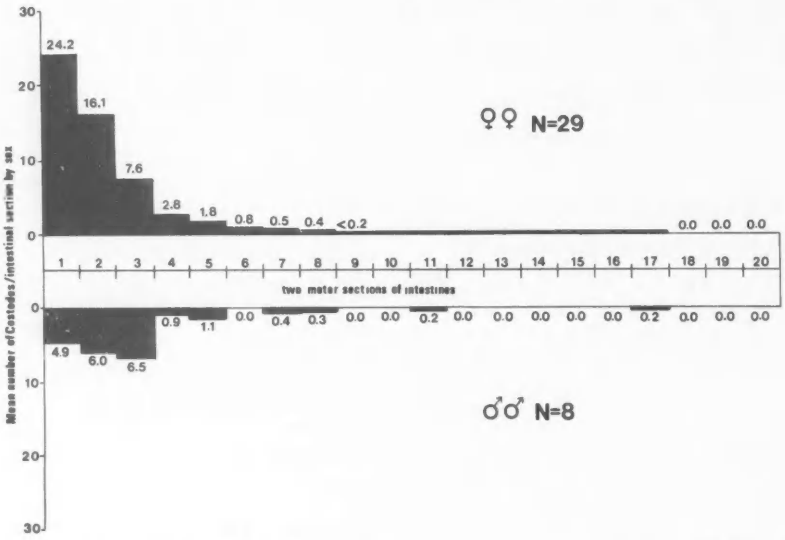

FIG. 1. Distribution of *Tetrabothrius forsteri* in the intestine of 37 *Lagenorhynchus acutus* stranded at Edmunds, Maine, USA. More than 95% of the tape worms were found in the first 8 m of intestine.

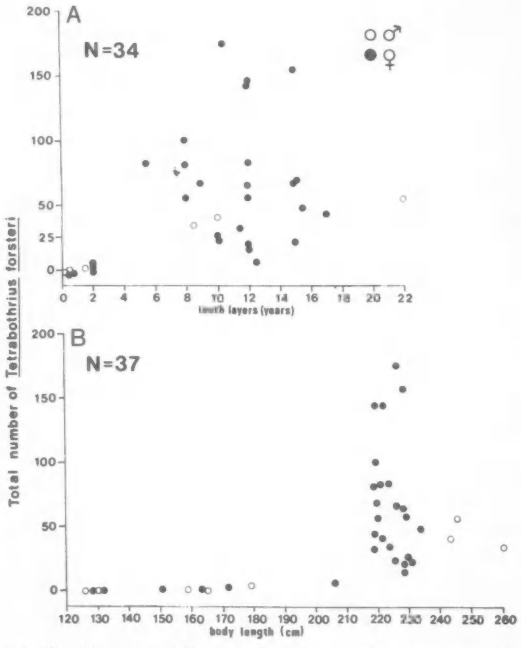

FIG. 2. Number of *Tetrabothrius forsteri* in relation to (A) age and (B) body length of 37 *Lagenorhynchus acutus* stranded at Edmunds, Maine, USA.

(Fig. 2). Male dolphins were less heavily infected than females.

Tetrabothrius forsteri and *O. laevicaecum* were first found in dolphins 151 and 165 cm long, respectively. This suggests that *L. acutus* becomes infected at about 1 yr of age when solid food is first consumed and weaning takes place. The gross and microscopic lesions associated with *O. laevicaecum* in the liver, pancreas, and hepatopancreatic ducts have been described by Geraci et al. (1978).

There was only one acanthocephalan noted in the Lingley dolphin sample, whereas five of six intestinal tracts examined from a previous stranding of *L. acutus* at Wellfleet, Massachusetts, in 1973 contained these parasites (J. R. Geraci personal communication). Although the sample size is small, there is a suggestion that *Bolbosoma* spp. may serve as useful biological tags to distinguish different feeding populations of *L. acutus*.

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Stomach Flushing: Effectiveness and Influence on Survival and Condition of Juvenile Salmonids

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MEEHAN, W. R., AND R. A. MILLER. 1978. Stomach flushing: effectiveness and influence on survival and condition of juvenile salmonids. J. Fish. Res. Board Can. 35: 1359–1363.

Hydraulic stomach flushing was an effective method to analyze relative food consumption and feeding habits of young salmonids. Coho salmon (*Oncorhynchus kisutch*) stomachs were most effectively flushed — 99% of invertebrate organisms (96% of weight of stomach contents); 92% of organisms (83% of weight) were flushed from cutthroat trout (*Salmo clarki*) stomachs, 90% of organisms (77% of weight) were flushed from rainbow trout (*S. gairdneri*) stomachs. Stomach flushing did not affect survival of juvenile coho salmon. A significant difference in change in mean condition factor between wild and hatchery fish was found after 30 d (greater change in wild fish), and a significant change was observed between the mean condition factors of control and treated hatchery fish. No difference was found between control and treated wild fish.

Key words: stomach flushing, juvenile salmonids, survival, condition, food habits

MEEHAN, W. R., AND R. A. MILLER. 1978. Stomach flushing: effectiveness and influence on survival and condition of juvenile salmonids. J. Fish. Res. Board Can. 35: 1359–1363.

Le curage hydraulique de l'estomac est une méthode efficace de détermination de l'importance relative des divers item consommés et des habitudes alimentaires des jeunes salmonidés. Les estomacs de saumons coho (*Oncorhynchus kisutch*) sont ceux qui sont le plus efficacement curés — 99% des invertébrés (96% du poids des contenus stomacaux); 92% des organismes (83% du poids) sont évacués des estomacs de truites fardées (*Salmo clarki*), 90% des organismes (77% du poids) sont évacués des estomacs de truites arc-en-ciel (*S. gairdneri*). Le curage stomacal n'affecte pas la survie des jeunes saumons coho. Nous observons une différence significative dans le changement de l'indice d'embonpoint de poissons sauvages et de poissons d'élevage après 30 jours (changement plus marqué chez les poissons sauvages), ainsi qu'un changement significatif de l'indice d'embonpoint de poissons témoins et de poissons d'élevage traités. Nous n'observons aucune différence entre les poissons témoins et les poissons sauvages traités.

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STUDIES of the food and feeding habits of fish are important in the study of aquatic ecosystems. Heretofore, food habit studies have required that many fish be killed for stomach removal and examination. In small streams with low fish populations, the removal of a few fish can seriously deplete the population. Thus, several methods have been developed to remove stomach contents without injuring the fish. These include (1) pressing on the stomach after a rigid tube has been inserted (White 1930), (2) injecting emetics (Markus 1932), (3) gastroscope viewing (Dubets 1954), (4) tube flushing (Seaburg 1957), (5) inserting forceps (Wales 1962), (6) back flushing intestines and stomach by placement of a pressure tube into the vent (Baker and Fraser 1976), and (7) syringe flushing (Aho 1976).

In this study a syringe flushing technique (Aho 1976) was used for examination of stomach contents of three salmonids: coho salmon, *Oncorhynchus kisutch*; cutthroat trout, *Salmo clarki*; and rainbow (steelhead) trout, *S. gairdneri*. We used this technique during the past 3 yr and found it to be reasonably harmless to fish. This paper addresses the following questions: (1) How are contents flushed from the fish stomachs correlated with total contents? (2) Are certain food items more likely to be flushed? (3) Is there a difference in food recovery between sizes, species, or stocks of fish? (4) How does handling affect survival and condition?

Methods — Effectiveness of stomach flushing — With a portable, battery-powered electroshocker, 51 juvenile rainbow trout, 51 cutthroat trout, and 53 young coho salmon were collected. The rainbow were collected from Canyon Creek, central Oregon, on July 29, 1975; cutthroat and coho were taken from Green River, a coastal stream in the Siuslaw National Forest, on August 1, 1975. Fish of a given species were collected in less than 2 h. They were then anesthetized with MS-222®, measured (fork length in millimetres), and the stomach contents were flushed into 56.7-g (2-ounce) collecting jars.

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Flushing was done by inserting a blunt 5.1-cm No. 18 hollow needle mounted on a 20-mL syringe filled with water through the mouth and esophagus into the cardiac stomach. When the water was forced into the stomach, the stomach contents were flushed back through the esophagus and mouth and via a funnel into the collecting jar. Usually one 20-mL injection was sufficient to flush out all the available contents, although larger fish sometimes required an additional injection. Care was taken to remove food items caught in the mouth. Before sealing collection jars, a 10% formalin solution and labels were added. Each fish was killed after stomach flushing and the stomach was removed and preserved in a second labeled collecting jar.

In the laboratory, food items flushed from the stomachs were identified (family or genus) and counted, and each total stomach sample was freeze-dried and weighed to the nearest 0.1 mg. Certain fragments such as head capsules represented one organism for numerical purposes; all fragments were included in sample weight. Food remaining in the preserved stomachs was handled identically. Regression analyses were run to establish relationships between (1) fish size and species and (2) amount of material flushed from stomachs. Covariance analysis was used to compare slopes and intercepts.

Effects on survival and condition — Wild and hatchery coho salmon juveniles were used in this phase of the study. Wild fish were collected from Green River in western Oregon; hatchery fish were obtained from the Oregon Department of Fish and Wildlife Hatchery on Fall Creek. Green River and Fall Creek are in the Alsea River drainage. All fish were collected by electroshocking, including those from the hatchery pond.

The fish were brought to the laboratory and held in separate large holding tanks for 3 d. During this time and throughout the experiment, they were fed 1.6-mm Oregon Pellets. Treatments at the beginning of the experiment consisted of electroshocking, measuring (fork length in millimetres), weighing (nearest 0.1 g), and finclipping all fish (treatment 1). Additional treatments were anesthetizing (treatment 2), and anesthetizing and stomach flushing (treatment 3). Control fish (treatment 1) were not additionally treated.

Three wild fish of each treatment (nine total) were placed into each of 10 glass jars (18-L). Each jar was individually aerated and supplied with fresh water. Hatchery fish were similarly arrayed in an adjacent unit of 10 jars.

TABLE 1. Number of invertebrates and weight of contents in stomachs of three species of salmonids.

Species	No.	Fork length (mm) Mean	Range	Stomach contents: Flushed No. organisms	Flushed Wt (g)[a]	Remaining No. organisms	Remaining Wt	Percent flushed[b] No. organisms	Percent flushed[b] Wt
Coho	53	65	52–92	596	639	6	24	99	96
Cutthroat	51	113	65–225	792	2336	70	446	92	83
Rainbow	51	139	93–232	845	2896	89	2259	90	77
Total	155	105	52–232	2233	5871	165	2729	93	85

[a]Freeze-dried weight.
[b]Percentage of stomach contents flushed was derived by calculating the percentage flushed from each fish and then finding the mean of these percentages.

The conditions in all jars were identical. Individual fish within each jar were identified by utilizing combinations of adipose, dorsal, upper, and lower caudal finclips. A total of 90 hatchery and 90 wild fish were used; mean lengths were 67.3 and 68.3 mm, respectively. Fish that died during this study were removed as soon as possible from the jars. After 30 d the surviving fish were again measured and weighed. Differences in survival and condition were tested by analysis of variance. Condition factor (K), a measure of relative robustness, was calculated as

$$K = \frac{W}{L^3} \times 10^5$$

where K is the condition factor, W is wet weight of fish in grams, and L is fork length of fish in millimetres.

Results and discussion — Effectiveness of stomach flushing — Flushing the 155 salmonid stomachs yielded a total of 2233 invertebrate organisms or 93% of the total number of prey (flushed and remaining) in the stomachs (Table 1). The total weight of stomach contents flushed from the three fish species was 5871 g or 85% of the total weight of contents (flushed and remaining).

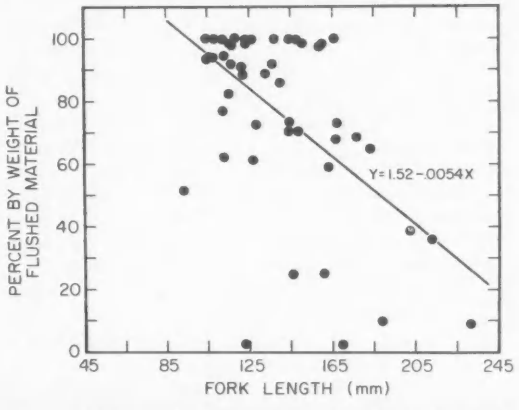

FIG. 1. Fish length vs. percent of material by weight flushed from stomachs of rainbow trout.

Examination of several specimens revealed that the pyloris of rainbow trout was longer and the diameter of the esophagus smaller in relationship to stomach size than in the other two species. These differences could inhibit the force of hydraulic current in the posterior stomach and decrease passage of contents out through the esophagus. Coho salmon were the smallest fish studied, cutthroat trout were intermediate, and rainbow trout were the largest. Food items found in the rainbow trout stomachs included more larger prey, such as small crayfish (*Astacus* sp.), sculpins (*Cottus* sp.), large stoneflies (e.g. *Acroneuria* sp), and large cased caddisflies such as *Dicosmoecus* sp. These larger, more rigid forms were much more difficult to flush from the stomachs; as a result, the percentage of material flushed from stomachs of the larger rainbows was smaller than that flushed from cutthroat and coho stomachs. Conversely, food items found in coho stomachs were much smaller and more pliable (e.g. Diptera larvae and Ephemeroptera nymphs) and were not as likely to become lodged in the stomach or esophagus.

Despite removal of caddisfly (Trichoptera) cases, snail shells, stones, and large wood pieces prior to weighing, much of the freeze-dried and weighed material in the stomachs of the three fish species was nondigestable matter. Small sand and wood particles, skeletons of hard-bodied insects, and densely packed sclerites from fully digested arthropods made up the bulk of the stomach contents. These fragments and partially digested material are densely compacted in the pyloris in preparation for passage through the intestine. As fish become larger and the muscle mass of the stomach increases, dislodgement of the contents from the pyloric stomach becomes more difficult.

A regression analysis of fish length vs. percent by weight of stomach contents flushed from rainbow trout (Fig. 1) established a negative linear correlation ($r^2 = .32$). This relationship did not exist for coho salmon ($r^2 = .05$), or cutthroat trout ($r^2 = .02$). This

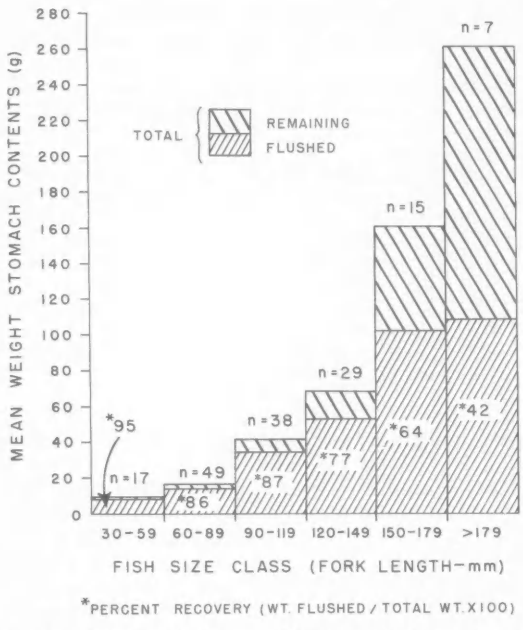

FIG. 2. Relationship between fish size and effectiveness of stomach flushing. Data are combined for coho salmon, cutthroat trout, and rainbow trout.

may be due to a higher percentage of fish having stomachs totally flushed (74 and 43%, respectively, compared to 25% for rainbow trout) and a preponderance of smaller fish (mean fork length 65 and 113 mm, respectively, compared to 139 mm for rainbow trout). These data are generally consistent with those obtained by Aho (1976) for cutthroat trout.

When data for the three fish species are combined, it appears that as fish size increases, the percent of material that is flushed from the stomach decreases (Fig. 2). This may be somewhat influenced, as mentioned earlier, by slight anatomical differences in the stomach and esophagus among the three species or by differences in the consistency of food items taken by each species.

Effects on survival and condition — Seventy-six (84%) of the hatchery fish and 78 (87%) of the wild fish survived the experiment. There was no significant difference in survival in either group between treated and control fish after 30 d (Table 2). Aho (1976) obtained similar results with cutthroat trout. Electroshocking, which causes severe trauma (Edwards and Higgins 1973), was probably the major cause of mortality, although measuring, weighing, and finclipping probably caused additional stress.

A significant difference in change in condition factor ($P \leq .01$) during the study was found between hatchery and wild fish when all treatments were considered together. The mean body condition of hatchery fish was poorer initially, but was less affected by treatment than that of wild fish. There was a significant difference ($P \leq .05$) in change in body condition between control and treated hatchery fish (Fig. 3). Mean condition factor was reduced to 96% (treatment 2) and 97% (treatment 3) of mean condition at the beginning of the experiment in the two treated groups; condition factor of control fish (treatment 1) was 103% of the original condition. No difference was found in change in body condition between control and treated wild fish. Considering the drastic change from their natural diet, i.e. from live invertebrates to Oregon Pellets, it is not surprising that they would do poorly. Any treatment differences that might have occurred would probably have been masked by effects of diet change.

TABLE 2. Survival of wild and hatchery coho salmon after 30 d (June 2 through July 1, 1975). Treatment 1 (control) – electroshocked; treatment 2 – electroshocked and anesthetized; treatment 3 – electroshocked, anesthetized, and stomach flushed. In addition, all fish were measured, weighed, and finclipped. For each treatment there were 30 fish in each group.

	Wild fish		Hatchery fish	
Treatments	No.	%	No.	%
1 (control)	27	90.0	25	83.3
2	25	83.3	23	76.7
3	26	86.7	28	93.3
Total	78	86.7	76	84.4

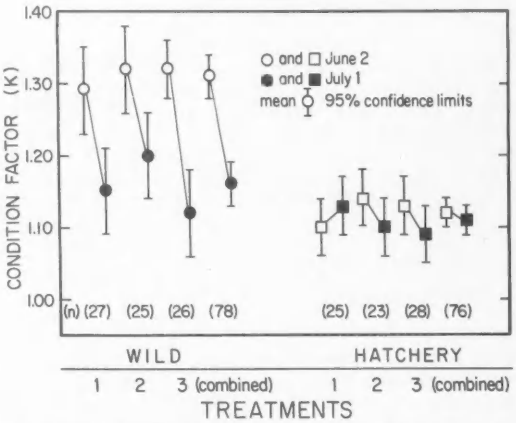

FIG. 3. Change in mean condition factor and 95% confidence limits of wild and hatchery coho salmon after 30 d (June 2 through July 1, 1975). Treatment 1 — electroshocked; treatment 2 — electroshocked and anesthetized; treatment 3 — electroshocked, anesthetized, and stomach-flushed.

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Use of Aniline Blue for Distinguishing Between Live and Dead Freshwater Zooplankton

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SEEPERSAD, B., AND R. W. CRIPPEN. 1978. Use of aniline blue for distinguishing between live and dead freshwater zooplankton. J. Fish. Res. Board Can. 35: 1363–1366.

It is possible to distinguish between live and dead freshwater zooplankton after 15-min immersion in an aqueous solution of aniline blue. Organisms physiologically dead at the time of staining are dyed blue, whereas living organisms remain unstained. This mortal staining technique has been repeatedly used in evaluating the survival of individuals belonging to all major groups of freshwater zooplankton. It has special application where the survival of large numbers of plankters must be determined either within a short interval, for successive intervals, or where conditions do not permit such evaluations immediately following collection or treatment. Stained samples preserved in formalin showed no evidence of leaching when stored at 4–15°C for several months. This technique has been used successfully for entrainment studies for power plants with once-through cooling systems.

Key words: freshwater plankton, zooplankton, phytoplankton, ichthyoplankton, dead–live determinations, mortal staining, power plant(s) entrainment, industrial monitoring

SEEPERSAD, B., AND R. W. CRIPPEN. 1978. Use of aniline blue for distinguishing between live and dead freshwater zooplankton. J. Fish. Res. Board Can. 35: 1363–1366.

Une immersion de 15 min dans une solution aqueuse de bleu d'aniline permet de distinguer entre le zooplancton d'eau douce vivants et le zooplancton mort. Les organismes physiologiquement morts au moment de l'immersion se colorent en bleu, alors que les organismes vivant ne prennent pas le colorant. On a utilisé cette technique à plusieurs reprises pour évaluer la survie d'individus appartenant à tous les principaux groupes de zooplanctontes d'eau douce. Elle est particulièrement utile lorsqu'il s'agit de déterminer la survie d'un grand nombre de planctontes, soit durant un court intervalle de temps pour plusieurs intervalles successifs, soit dans les cas où les conditions rendent impossibles de telles déterminations immédiatement après la collecte ou le traitement. Les échantillons colorés conservés dans de la formaline durant plusieurs mois à des températures de 4 à 15°C ne montrent aucun signe de décoloration. Cette technique a été utilisée avec succès dans des études d'entraînement dans les centrales électriques avec systèmes refroidisseurs monocycliques.

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THERE has been a need for a rapid and efficient technique to distinguish live from dead individuals among freshwater plankton. An effective vital or mortal staining technique is a useful tool for such distinctions, and one which also reduces prolonged handling stress and enables postponement of observations to a convenient time. Vital and mortal staining techniques have been reported for vegetable tissues, microbes, mammalian gametes, and marine plankton (Campbell et al. 1956; Gaff and Okong'O-Ogola 1971; Dressel et al. 1972; Crippen and Perrier 1974; Schwantes 1975). The vital staining techniques using neutral red described by Dressel et al. (1972) and Crippen and Perrier (1974) for marine zooplankton failed to produce parallel results with their freshwater counterparts, even with modifications to the dye concentrations and staining periods. Physiological processes may be the factor governing the differences in response to neutral red between freshwater zooplankton and their marine relatives.

The purpose of this paper is to describe a new mortal staining technique to distinguish between live and dead freshwater zooplankton.

Materials and methods — Zooplankton samples used for the development of this staining technique were collected with a no. 20 (76-μm mesh) plankton net along the northern shore of Lake Ontario in the vicinity of Toronto, Ontario.

Paired groups of live and "heat-killed" samples (killed at 55°C) were transferred into separate 5 cm dia sieves and subsequently immersed in aniline blue solution at ambient temperature for 5, 10, 15, and 20 min using concentrations of 0.5, 2.3, 5.0, and 7.5 g · 100 mL^{-1} of deionized tap water. The aniline blue used for these experiments was manufactured by BDH Chemicals, Poole, England (Lot 320551/5151). Sieves were constructed from short pieces of plexiglass tubing sealed at one end with 76-μm mesh nitex. Care was taken to ensure complete immersion of the test organisms in the dye solution. Samples were removed at the end of their respective staining times, washed with tap water to remove excess dye, and observed before and after preservation. Samples were preserved with sufficient formalin to make a final concentration of 10%.

Acidification with a buffered acid following preservation was found to facilitate the distinction between organisms that were alive or dead at the time of staining, especially for smaller organisms such as nauplii and rotifers. Sufficient stock solution containing equal volumes of 1 M acetic acid and 1 M sodium acetate was added to bring the pH of the preserved sample to 6.5. Samples were examined 15 min following acidification.

The effectiveness of aniline blue to distinguish between live and dead larval fish was tested on samples of white suckers (*Catostomus commersoni*) collected from Lake Ontario and splake (*Salvelinus namaycush* × *S. fontinalis*) obtained from the Ministry of Natural Resources at Wiarton, Ontario. Larval fish staining tests were similar to those described for zooplankton; however, only a concentration of 5.0 g of dye · 100 mL^{-1} of freshwater and a 15-min staining period were employed. To test for mortal staining, larval fish were killed by thermal, chemical, and mechanical treatments. These treatments included heat shock at 50 or 35°C, coldshock at 0.5°C, 10% formalin, 1% sodium cyanide, and cerebral damage inflicted by forceps.

Results and discussion — "Heat-killed" zooplankton samples exposed to aniline blue at concentrations of 0.5 and 2.3 g · 100 mL^{-1} were ineffectively stained at 5-, 10-, 15-, and 20-min immersion times. The best staining results were obtained with a 15- or 20-min immersion at a concentration of 5 and 7.5 g aniline blue · 100 mL^{-1} of water. "Heat-killed" specimens of all zooplankton groups observed after this immersion time appeared conspicuously blue, whereas the accompanying fresh plankton samples (composed of live and dead organisms) contained both stained and unstained specimens. Observations made prior to preservation showed all unstained individuals to be alive and all stained individuals to be dead. "Heat-killed" individuals stained with these latter concentrations for 5 and 10 min were inconsistently stained. The concentration of 5.0 g · 100 mL^{-1} for 15 min was selected for routine live–dead evaluations to avoid any possible adverse effects at higher concentrations and longer staining periods. Similar results were obtained for zooplankton popula-

TABLE 1 Percent mortality of natural and heat-killed zooplankton populations as determined by 15-min immersion in aniline blue (5 g of dye · 100 mL^{-1} of freshwater). Sample size is in parentheses.

	Ambient temp (°C)	Natural			Heat killed		
		Copepoda	Nauplii	Cladocera	Copepoda	Nauplii	Cladocera
				1976			
Oct. 21	7.1	10.0 (191)	59.0 (58)	15.0 (287)	100.0 (400)	100.0 (136)	100.0 (250)
Nov. 3	5.9	1.0 (303)	19.0 (349)	9.0 (131)	100.0 (175)	100.0 (200)	100.0 (125)
Dec. 2	5.2	20.0 (112)	17.0 (144)	30.0 (40)	100.0 (129)	100.0 (110)	100.0 (50)
				1977			
Jan. 20	4.8	27.0 (270)	61.0 (69)	—	100.0 (169)	100.0 (34)	—
Mar. 2	1.0	26.0 (509)	74.0 (469)	3.0 (33)	100.0 (301)	100.0 (350)	100.0 (25)
April 7	5.0	11.0 (257)	41.0 (406)	—	100.0 (122)	100.0 (102)	—
April 22	4.8	9.7 (924)	—	—	100.0 (512)	—	—
Sept. 15	16.0	7.0 (134)	10.0 (200)	—	100.0 (212)	100.0 (176)	—
Sept. 22	15.0	17.0 (173)	—	0.0 (21)	100.0 (320)	100.0 (200)	100.0 (25)

TABLE 2. Dominant zooplankton species present in experimental samples.

Zooplankton	
Copepoda	Cladocera
Cyclops bicuspidatus thomasi	*Alona affinis*
Diaptomus ashlandi	*Bosmina coregoni*
D. minutus	*B. longirostris*
D. oregonensis	*Ceriodaphnia lacustris*
D. sicilis	*Chydoras sphaericus*
Eurytemora affinis	*Diaphanosoma leuchtenbergianum*
Mesocyclops edax	*Daphnia galeata mendotae*
Tropocyclops edax	*D. longiremis*
Nauplii	*D. magna*
	D. retrocurva
	Eubosmina coregoni
	Leptodora kindtii
Rotifera	Rotifera
Argonotholca foliacea	*Notholca acuminata*
Asplanchna priodonta	*N. laurentiae*
Brachionus calyciflorus	*N. squamula*
Euchlanis dilatata	*Ploesoma truncatum*
Keratella cochlearis	*Polyarthra dolichoptera*
K. crassa	*Synchaeta lakowitziana*
K. earlinae	*S. pectinata*
K. quadrata	*Trichocera multicrinis*
Lecane bulla	*T. porcellus*

tions collected from several ambient temperatures (Table 1). These results showed that the staining effect is temperature independent. The high mortalities among populations reported in Table 1 for some groups were consistent with the percent mortalities encountered for concurrent in situ field studies. These data probably reflect natural seasonal mortality among zooplankton populations and/or sampling mortality.

It is recommended that a "heat-killed" sample be stained with each series of survival determinations to ensure the effectiveness of the dye in staining dead organisms. For zooplankton, the best results were obtained from samples brought quickly to 55°C and then allowed to cool at room temperature for 1 h. Some groups or developmental life stages of organisms "heat-killed" at temperatures greater than 59°C were found to contain some unstained individuals. These inconsistencies in staining may be due to a total denaturation of protein at higher temperatures. "Heat-killed" samples, therefore, should not be heated above 59°C. Caution should be used in interpreting data resulting from samples stained immediately following treatment. Plankters may be in a moribund condition from a given stress; however, they may remain physiologically alive for some time, in which case they will not pick up the stain, or they may partially stain. Partial staining has also been noted in healthy female calanoid copepods in the vicinity of spermatophores. Staining in this region should not be misinterpreted. A list of the dominant zooplankton species occurring among the populations

TABLE 3. Time between treatment and complete staining (physiological death) of larval white sucker (*Catostomus commersoni*) from an ambient temperature of 12°C.

Treatment	Time to dye absorption (death)	Comments
50°C	15 min	100% staining
35°C	4 h	100% staining
0.5°C	4 h	100% staining
Cerebral damage	6 h	Only damaged areas picked up the dye before this time.
1% sodium cyanide	10 min	100% staining
10% formalin	10 min	100% staining
Untreated (control)	—	No staining[a].

[a]After repeated 15-min immersions during a 12-h period, arval fish were stressed due to handling and stained light blue.

tested is presented in Table 2. Larval fish killed by formalin and sodium cyanide became fully stained when immersed in aniline blue solution 10 min after treatment. However, those treated by heat shock at 50°C stained after 15-min immersion, whereas those treated at 35°C or with coldshock at 0.5°C required 4–6 h to become fully stained. Larval fish subjected to cerebral damage were fully stained after 6 h. Although these latter larval fishes were in a moribund state at the time of cerebral damage, physiological death only became evident through complete staining 6 h after treatment (Table 3). Myomeres among dead larval fish and scale annuli were enhanced providing aids for taxonomic and aging purposes.

"Heat-killed" copepod eggs appeared to stain inconsistently with aniline blue. Dressel et al. (1972) noted similar results with eggs of marine copepod species (*Acartia tonsa* and *Eurytemora affinis*) using neutral red. Although no laboratory experiments were performed, fish eggs occurring in field collections appeared to respond to aniline blue in a similar manner to the larval fish and zooplankton.

Experiments were repeated on major groups of live organisms for possible adverse effects from using aniline blue (Table 4). No apparent toxic effects were noted after 15 min in the dye solutions; however, results from staining periods longer than 15 min suggested adverse effects to some organisms. Reexamination of preserved samples after several months of storage showed no evidence of leaching at temperatures between 4 and 15°C.

Dye ingestion is useful in evaluating sublethal stresses such as those caused by temperature, mechanical effects, and toxic effects. Dressel et al. (1972) used the ingestion of carmine red as a criterion for distinguishing between live and dead marine copepods (*A. tonsa* and *E. affinis*). Our results showed that healthy daphnia (*Daphnia magna*) readily ingested carmine red (Dressel et al. 1972), while stressed animals did not. However, those cladocerans that later recovered did ingest the

TABLE 4. Zooplankton groups investigated for possible adverse effects of aniline blue dye after 15-min immersion.

Zooplankton	No. replicates	Total no. individuals	Comments
Copepoda	5	35	All alive with dye in gut.
Cladocera	5	30	All alive with dye in gut.
Nauplii	3	42	All alive with dye in only a few guts.
Rotifera	3	19	All alive with dye in only a few guts.
Larval fish (splake and white sucker)	3	30	All alive.

dye. Similar ingestion patterns were noted among various groups in field samples immersed in aniline blue solution. This ingested dye did not diffuse into the tissues following preservation.

Casual observations made of phytoplankton occurring in stained zooplankton samples showed nearly total staining in "heat-killed" control samples and both stained and unstained cells of the same species in live samples. Although it was not the intent of this study to investigate the response of phytoplankton to mortal staining with aniline blue dye, the observations suggested that phytoplankters may respond similarly to zooplankters after immersion in the dye solution.

Aniline blue has been successful in distinguishing live from dead plankton in entrainment studies at thermal generating stations, following laboratory entrainment simulations, and in the vicinity of industrial outfalls. Mortality of affected organisms can easily be evaluated immediately following an experiment or delayed to a more convenient time.

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Toxicological Comparison of Natural and Cultured Populations of *Acartia tonsa* to Cadmium, Copper, and Mercury

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SOSNOWSKI, S. L., AND J. H. GENTILE. 1978. Toxicological comparison of natural and cultured populations of *Acartia tonsa* to cadmium, copper, and mercury. J. Fish. Res. Board Can. 35: 1366–1369.

Cultured *Acartia tonsa* manifested a reproducible toxicological response through six generations. There were no statistically significant differences in the responses of F_1 and F_6 generations to cadmium, copper, and mercury. Cultured and field populations (parental) exposed to cadmium did not manifest statistically different response patterns. The response

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variability of cultured populations was less than that of field populations. The 96-h LC50 concentrations for cultured (F_1) populations were 90 μg Cd/L, 31 μg Cu/L, and 10 μg Hg/L. The presence and abundance of ova and unicellular glands in the circumesophogeal region were used as criteria for evaluating the nutritional condition of test populations.

Key words: marine, metals, bioassay, culture, calanoid copepods, toxicity, histology, filial generations

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Acartia tonsa en culture a une réaction toxicologique reproductible durant six générations. Il n'y a pas de différences statistiquement significatives dans les réactions des générations F_1 et F_6 au cadmium, au cuivre et au mercure. La façon dont les populations cultivées et sauvages (parentales) exposées au cadmium réagissent n'est pas statistiquement différente. La variabilité des réponses des populations cultivées est moindre que celle des populations sauvages. Les CL-50 après 96 h pour les populations cultivées (F_1) sont de 90 μg Cd/L; 31 μg Cu/L; et 10 μg Hg/L. Comme critères d'évaluation de la condition alimentaire des populations d'essais, nous avons utilisé la présence et l'abondance des oeufs et les glandes unicellulaires dans la région circumoesophagienne.

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HISTORICALLY, marine bioassay organisms were collected or spawned from field populations. This imposes restraints upon the researcher in terms of continuous availability of assay organisms in large quantities, nutritional and genetic uniformity, variability in age of the organisms, and incidence of disease. These restraints are particularly important in programs where continuous or repetitive testing is required, or a comparative data base is being compiled.

Static and continuous flow systems have been used successfully to produce successive generations of calanoid copepods for toxicological studies (Gentile et al. 1974). These meet criteria necessary for all organisms used for toxicological purposes. First, and most importantly, cultured species must have a nutritional history that is both qualitatively and quantitatively similar to those occurring in their natural environment so that their response will not be biased by nutritional condition. Second, species cultured for several generations must have reproducible responses to stress; and third, they must manifest a sensitivity similar to field populations.

The objectives of this study are (1) to examine the response reproducibility and sensitivity of the calanoid copepod *Acartia tonsa* to three metal pollutants, (2) to compare responses of cultured and field populations, and (3) to employ histological techniques for the assessment of the nutritional state of test organisms. Implicit in this approach is that both response reproducibility and sensitivity reflect the nutritional condition of the organisms.

Materials and methods — Test organisms (*Acartia tonsa*) were collected August 13, 1973, from a depth of 1 m with a 0.5-m (no. 10 mesh; 150 μM) plankton net in the Pettaquamscutt River estuary, Narragansett, R.I. Three- to 5-min plankton tows were taken from the Bridgetown Bridge. Current speed was approximately 0.5 m/s. Ambient water temperature and salinity were 24°C and 10 ‰, respectively. Tow material was diluted fivefold with river water in insulated containers and returned to the laboratory within 30 min. Five, 200-mL samples of tow material were transferred to 190 × 100-mm Pyrex crystalizing dishes and diluted to ~2000 mL with filtered (0.45 μM) river water. These samples were incubated overnight at 20°C and 1000 lx on a 14L:10D cycle. Adult female *A. tonsa* were then sorted using standard taxonomic criteria (Wilson 1932) and placed in generation cages. After 24 h, eggs were collected and used to start two mass cultures. The details of the culture procedure are given in Gentile et al. (1974).

Acartia tonsa feeds in the water column; therefore, food organisms were maintained in the water column by stirring at ≤25 rpm. Previous culture systems for *A. tonsa* failed to keep food available (Zillioux and Lackie 1970; Heinle 1969). A second consideration was the quality and quantity of food organisms. *Acartia tonsa* cultures were often fed unrealistic algal food densities poor in the naturally occurring food organisms, planktonic diatoms, which are more difficult to culture (Zillioux and Wilson 1966; Wilson and Parrish 1971; Heinle 1969). The cultured filial generations in this study were fed four algal species. *Isochrysis galbaena* (5.0×10^6/L) and *Rhodomonas baltica* (3.0×10^6/L) have been used historically for culturing copepods. *Skeletonema costatum* (5.0×10^6/L) and *Thalassiosira pseudonana* (7.0×10^6/L) provide the natural staple diet for the genus *Acartia*. *Skeletonema costatum* (6–10 μM) is of a size range preferred by adults, while *T. pseudonana* (2–4 μM) is suitable for naupliar stages. The total algal cell density (2.0×10^7/L) reflects the maximum algal cell density in Narragansett Bay (Smayda 1957, 1973; Pratt 1959).

Additional collections of *A. tonsa* were made as described above from lower Narragansett Bay. *Acartia tonsa* were held at 20°C and 30 ‰ for 24 h and fed (as above) before testing.

Histological examinations were made on all collected and cultured populations according to methods of Barszcz and Yevich (1976).

TABLE 1. Analysis of cadmium toxicity (LC50 in $\mu g\ Cd^{+2}/L$) to the calanoid copepod *Acartia tonsa* after 96-h exposure.

Probit analysis	P[a]	F_1[b]	F_6[c]	B[d]
Mean LC50	337	90	220	122
Upper limit (95%)	530	135	313	341
Lower limit (95%)	184	58	138	60
Analysis of variance				
Source	df	SS	MS	F
Total	74	96 029		
Concentration	4	75 850	18 962	106.9[e]
Population	4	3 801	950	5.3[e]
Error	50	8 862	117	

[a]Parental stock; [b]first filial generation; [c]sixth filial generation; [d]bay population; [e]F .95(4, 50) = 2.56.

TABLE 2. Analysis of copper toxicity (LC50 in $\mu g\ Cu^{++}/L$) to the calanoid copepod *A. tonsa* after 96-h exposure.

Probit analysis	F_1[a]	F_6[b]	B[c]	
Mean LC50	31	55	17	
Upper limit (95%)	44	76	29	
Lower limit (95%)	21	36	9	
Analysis of variance				
Source	df	SS	MS	F
Total	89	105 264		
Concentration	4	80 872	20 218	131.5[d]
Population	3	7 316	1 463	9.5[e]
Error	60	9 225	154	

[a]First filial generation; [b]sixth filial generation; [c]bay population; [d]F .95(60) = 2.52; [e]F .95(3, 60) = 2.80.

Static bioassays were performed on selected filial generations from cultures (F_1, F_2, F_6), on the parental *A. tonsa* field population from Pettaquamscutt River (P), and on field populations of *A. tonsa* (B) collected from Narragansett Bay. Forty-five organisms, as three replicates of 15, were exposed in each of five toxicant concentrations and a control. Duration of the experiment was 96 h. Dead animals were removed and recorded daily. Exposure conditions were 20°C and 10‰ for the P and F generations of *A. tonsa*; 20°C and 30‰ for B populations. Salinity differences reflect those found in collection sites. Light was programmed for 14L:10D for all *A. tonsa* studies to coincide with conditions in the field at the time of collection.

Stock solutions of cadmium, copper, and mercury were prepared by dilution atomic absorption primary standards (Alpha Analytical Lab., N.J.) in deionized water (18 Mohm at about 20°C). Dosages are reported as nominal values.

To obtain the toxicant LC50 and the upper and lower 95% confidence interval (Finney 1971), mortality response patterns were first analyzed by probits. The mortality response patterns of the various source populations were also analyzed by two-way analysis of variance. When necessary, the treatments' sums of squares were partitioned to identify which populations were different (Snedecor and Cochran 1967).

Results and discussion — Studies using cadmium were the most pertinent because, in this series, comparison testing with the parental (field) population was possible. The bioassay data for 96-h exposure were tested with analysis of variance (Table 1) to determine whether the toxicological responses observed for each source population to cadmium were the same. There were significant ($P < .05$) differences in responses due to population source and due to toxicant concentration. There were no differences ($P \geq .05$) between the P, F_1, and F_6 populations, or between P, F_1, and B populations, but significant differences ($P < .05$) occurred between the F_6 and B populations.

Graphical estimation of the F_2, 96-h LC50 resulted in a value of 190 $\mu g\ Cd^{+2}/L$ which is within the range of both F_1 and F_6 generations and bay (B) data. The difference between the upper confidence interval and the mean LC50, divided by the mean LC50, produces a simple index of response variability within the assay population. These values are $P = 57\%$; $F_1 = 50\%$; $F_6 = 42\%$; $B = 179\%$; which indicate that the B population has a greater intrinsic variability than the cultured or parental populations.

TABLE 3. Comparison of LC50 values ($\mu g\ Hg^{+2}/L$) for filial generations of *A. tonsa* exposed to mercury.

Organism (population)	Exposure time (h)			
	24	48	72	96
F_1	17.5	17	13	10
F_2	20	19	17	14
F_6	22	19	16	15

Acartia tonsa F_1, F_2, F_6, and B populations were exposed to copper. Analysis of variance of toxicity data to F_1, F_6, and B populations indicates significant ($P < 0.05$) differences in their responses (Table 2). Filial generations F_1 and F_6 were not statistically different from each other. However, the *A. tonsa* B population was significantly different from filial populations. The F_2 population had a 96-h LC50 of 64 $\mu g/L$. Using the response variability index described previously, we found that the cultured populations ($F_1 = 38.5\%$, $F_6 = 38\%$) had less variability than the bay population ($B = 71\%$).

Data for mercury (Table 3) was not amenable to probit analysis because the response patterns were not suitably graded over time. That is, mercury toxicity had reached its maximum effect within 24 h after exposure was initiated. Since exposures were static, it is possible that mercury was no longer available in a biologically active form.

The toxicological data for cadmium and copper indicate that there is no statistically significant difference in cultured populations after six filial generations. Though not analyzed statistically, the mercury data from filial

generation bioassays show very little response variation. Comparison of field and cultured populations did, however, reveal inconsistencies.

The agreement between the parental (field) and successive filial generations exposed to cadmium suggests that these populations were nutritionally similar at the time of testing. In both cadmium and copper studies, the only statistically significant differences between source populations involved the *A. tonsa* bay populations. This could be the result of salinity differences in the test conditions, the lack of acclimation with feeding before testing, and the high response variability characteristic of the bay populations. The single point sample represented by our field populations reflects only one set of nutritional and environmental conditions in a constantly changing continuum. The culture conditions were chosen to represent optimal field conditions. Therefore, unless the tested field populations corresponded to these conditions, agreement would be unlikely. Ideally, bioassays should be conducted on a series of natural populations to account for changes in food (quality and quantity) and age structure of test populations. Such studies would describe the expected natural response variability with which one could confidently compare data derived from cultured populations.

Histological examinations were performed on *A. tonsa* populations used for toxicological studies. The filial generations (F_1, F_2, and F_6) examined had abundant, normally developed ova and many unicellular esophogeal glands. The field populations (P, B), however, had few ova and a paucity of unicellular glands. Wilson and Parrish (1971) reported that egg production in *A. tonsa* is directly related to algal food density and, by inference, to nutrition. Gonzalez et al. (1975) suggested that the abundance of unicellular glands in the circumesophogeal region is indicative of nutritionally sound organisms. This hypothesis is based on the observation that starved *A. tonsa* populations show a progressive diminution of both ova and unicellular esophogeal glands. The abundance of ova and unicellular glands in our cultured populations affirms that both the quantity and quality of the algal diet used in this study (Gentile et al. 1974) provided adequate nutrition for *A. tonsa*.

The general lack of both ova and unicellular glands in our field populations is not unexpected when one considers the dynamic nature of phytoplankton–zooplankton cycles. The histological data indicate the field populations were nutritionally marginal compared with cultured populations. This is reflected in the greater variability in responses to toxicants demonstrated by the field populations.

We conclude that the culture methods employed in this study provide a nutritionally and physiologically consistent research organism. This enhances the confidence in laboratory derived data for this species and its application to natural populations.

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Capture Probability: The Role of Zooplankter Escape in the Selective Feeding of Planktivorous Fish

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The conditional probabilities ($P3$) of being captured by a simulated fish-suction intake ranged from $P = 0.76$ to $P = 0.96$ for cladocerans and from $P = 0.07$ to $P = 0.28$ for copepods. Gizzard shad (*Dorosoma cepedianum*) feeding experiments confirmed that these differential capture probabilities can result in an apparent planktivore feeding selectivity for cladoceran zooplankters.

Key words: capture probability, zooplankter escape, planktivore feeding selectivity, gizzard shad

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Les probabilités conditionnelles ($P3$) de capture par l'appareil à succion simulé d'un poisson varie de $P = 0.76$ à $P = 0.96$ pour les cladocères et de $P = 0.07$ à $P = 0.28$ pour les copépodes. Des expériences d'alimentation effectuées avec des aloses à gésier (*Dorosoma cepedianum*) confirment que ces probabilités de capture différentielles peuvent résulter en une apparente sélection des cladocères par des poissons planctivores.

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A PREDATOR-PREY interaction can be broken down into a sequence of four events: encounter, attack, capture, and ingestion (Gerritsen and Strickler 1977). We assume that the four events are independent. Therefore, the probability that a prey will be eaten is the product of the conditional probabilities of those four events ($P1 \times P2 \times P3 \times P4$). Evaluation of these probabilities reveals each event's role in a predator's selective feeding.

Because studies found that predation by planktivorous fish can shift the freshwater zooplankton community toward species with smaller body sizes (Hrbacek et al. 1961; Brooks and Dodson 1965), most studies of planktivore feeding have focused on the role of the encounter and attack events in planktivore size-selectivity (Werner and Hall 1974; Confer and Blades 1975; O'Brien et al. 1976). Although Brooks and Dodson (1965) provided evidence that prey escape abilities altered the size-selective impact of planktivores, only a few studies have investigated the role of the capture event in planktivore feeding (Confer and Blades 1975; Rosenthal 1969; Starostka and Applegate 1970). The objective of this study was to examine experimentally the role of the capture event in determining the feeding selectivity of planktivorous fish. We simulated a fish's mechanism for capturing zooplankters with a siphon and used the simulator to determine the capture probability for five genera of zooplankters. The potential role of these capture probabilities in planktivore feeding was confirmed in laboratory feeding experiments with gizzard shad (*Dorosoma cepedianum*).

Capture probability is also a function of predator capture mechanisms. Most teleostean fish capture prey

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using a suction created by expansion of the buccal and opercular cavities (Alexander 1970). The buccal suction-pump inhales a volume of water, creating a flow field in front of the mouth similar to flow into a pipe (Alexander 1967). Flow field characteristics depend on the size of the mouth opening, the volume of the buccal cavity, and the rate of buccal cavity expansion (Nyberg 1971). Buccal cavity volumes usually range from 5 to 8 mL/100 g body weight (Alexander 1970). Buccal cavity expansion times measured as the time between mouth opening and closure range from 0.04 s for largemouth bass (*Micropterus salmoides*) (Nyberg 1971) to 0.85 s for the anabantoid fish (*Helostoma temmincki*) (Liem 1967).

Materials and methods — We simulated a fishlike intake using a siphon system controlled by a solenoid valve (Drenner 1977). In 0.4 s, 10 mL of water were inhaled through a glass tube of 1.0 cm inside diameter, the end of which was centered in an 80-L aquarium. To determine the capture probability of nonmotile particles we used neutrally buoyant bubbles of xylene and *n*-butyl phthalate colored with pertoleum dye; these bubbles were similar to zooplankters in size and specific gravity. We then determined the capture probability for freshly heat-killed zooplankters (*Chaoborus* sp., *Daphnia pulex*, and *Diaptomus pallidus*). By comparing these capture probabilities we could evaluate how particle or organism shape affects evasion. Finally, we determined the capture frequency for live zooplankters swimming freely in the aquarium. To reduce problems of spatial resolution we attempted to capture only particles and zooplankters that were directly in front of the tube. The distance between the opening of the tube and the center of the particle or animal was measured using a horizontal cathetometer (Drenner 1977).

To test the results of the capture experiments, we determined the relative feeding rates of gizzard shad on a mixture of zooplankters in laboratory experiments and compared those feeding rates with the capture probabilities obtained from the capture experiments. For each experiment 31–38 gizzard shad ranging from 6.9 to 9.9 cm in standard length were placed in a plastic swimming pool containing 120–150 L of water. Invertebrate predation and other effects were monitored in a control pool of the same volume without shad. An experiment began when freshly caught zooplankters were poured into the pools. Following a thorough mixing of the pools, zooplankters were sampled by quickly lowering a clear plexiglass tube 6.9 cm diam onto a rubber stopper which had been randomly placed on the pool bottom. The tube, which now contained a column of water, was removed from the pool and the contents preserved in 10% formalin. One to three such samples were made at each sampling time depending on how frightened the shad became. Initial zooplankter species concentrations ranged from 13.5 to 376.0 organisms/L. Feeding trials lasted from 1 to 13.5 h. Five trials were conducted in diffused light and three in the dark. The movement of the fish kept the pools well mixed.

Results — Capture experiments — Figure 1A shows the results of 200 capture trials of nonmotile bubbles and heat-killed zooplankters. The capture frequency of the dead zooplankters was identical to that of the bubbles and thus the shape of the particle or dead organism appears not to be important in evasion of flow fields.

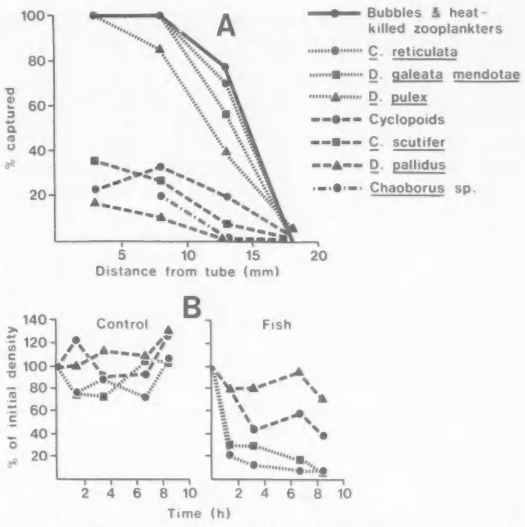

FIG. 1. A, Capture frequencies of bubbles and zooplankters in front of siphon tube; B, one of shad feeding experiments showing changes in zooplankter densities in the control pool and fish pool (from Drenner 1977).

Live zooplankters were able to evade the suction field. These animals respond with negative or positive rheotaxis to acceleration and shear fields (Strickler 1975). They perceive hydrodynamic disturbances with their mechanoreceptors (Strickler 1975) and then evade capture at speeds shown to be 6 cm/s for *Daphnia* (Cushing 1955), 30–50 cm/s for *Cyclops scutifer* (Strickler 1975), and 147 cm/s for *Diaptomus kenai* (Swift and Fedorenko 1975).

The capture frequencies of the cladocerans *Ceriodaphnia reticulata* and *Daphnia galeata mendotae* (Fig. 1A) were not significantly different than the capture frequency for the bubbles and dead zooplankters using a G-test (Sokal and Rohlf 1969). However, the larger *D. pulex*, which had higher cruising speeds, could escape significantly better ($P < 0.05$) (Fig. 1A). The capture frequencies of the cyclopoid copepods (composed of *Cyclops* sp. and *Mesocyclops* sp.), *C. scutifer*, *Diaptomus pallidus*, and *Chaoborus* sp. were also significantly different than the capture frequency for the bubbles and dead zooplankters ($P < 0.005$) (Fig. 1A).

We define the conditional probability ($P3$) for a live zooplankter as the ratio between the areas under the capture frequency curves for (a) the live zooplankter and (b) the nonmotile bubbles and dead zooplankters. These calculated capture probabilities ($P3$) were highest for the cladocerans *Ceriodaphnia reticulata* ($P3 = 0.96$), *Daphnia galeata mendotae* ($P3 = 0.92$), and *D. pulex* ($P3 = 0.76$); intermediate for cyclopoid copepods ($P3 = 0.28$) and *Cyclops scutifer* ($P3 = 0.24$);

and lowest for *Diaptomus pallidus* ($P3 = 0.07$) and *Chaoborus* spp. ($P3 = 0.09$).

Fish feeding experiments — The significance of the capture event depends on the type of planktivore. For those fish that do not visually select individual prey, such as filter feeders feeding at night, capture probability ($P3$) is the dominant event determining selectivity. Encounter ($P1$) and attack ($P2$) probabilities are proportional to zooplankter densities. Because the probability of ingestion ($P4$) is determined by the relative size of the prey and the distance between gill rakers, $P4$ is similar for prey of similar size. Thus the diet of nonvisual-feeding planktivores on similar-sized prey will be determined primarily by differential capture probabilities. Measurements of distance between gill rakers show that shad filter particles larger than 0.1 mm with 100% efficiency (Drenner 1977). Therefore, shad feeding rates on crustacean zooplankters should be determined by differential capture probabilities. Gizzard shad above 3.0 cm are omnivorous (Cramer and Marzolf 1970), feeding on phytoplankton, zooplankton, and detritus by filtering water inhaled using the buccal-suction pump.

Apparent fluctuations of zooplankter numbers in the control and fish pools (Fig. 1B) were probably caused by sampling variability resulting from using only a minimal number of samples to avoid frightening the shad. This tube sampling is more efficient than suction sampling (Janssen 1976) but is still not 100% efficient (Szlauer 1964).

As predicted from the calculated capture probabilities, cladoceran densities decreased more rapidly than copepod densities in the fish pool (Fig. 1B). Shad feeding rate constants (k) were calculated using Dodson's procedure (Dodson 1975) with the dimensions of k being litres per hour. Feeding rate constants increase linearly with capture probability (Drenner 1977). Feeding rate constants were lowest on *Diaptomus* spp. (mostly *D. pallidus*) ($xk = 0.67$), intermediate on cyclopoids ($xk = 1.37$), and highest on *Daphnia* spp. (*D. galeata mendotae* and *D. pulex*) and *Ceriodaphnia reticulata* ($xk = 3.60$ and 3.00, respectively). The feeding rate constant for copepod nauplii was also high ($xk = 4.01$). Nauplii of *Artemia* have previously been found to have a probability of capture by herring as high as 100% (Rosenthal 1969). Nonparametric multiple comparison by the simultaneous test procedure (Sokal and Rohlf 1969) showed shad feeding rate constants for *Diaptomus* spp. differ ($P < 0.05$) from the feeding rate constants for *C. reticulata*, *Daphnia* spp., and copepod nauplii.

Discussion — These results show that differential capture probabilities result in an apparent feeding selectivity for cladoceran zooplankters by nonvisual-feeding planktivores. Field studies of the feeding of omnivorous gizzard shad (Cramer and Marzolf 1970) and adult bigmouth buffalo (*Ictiobus cyprinellus*), which also apparently do not visually select individual food items (Starostka and Applegate 1970), have suggested such feeding selectivities. These results may also explain the mechanism by which the sardine *Limnothrissa miodon* selects the cladoceran *Bosmina longirostris* over the copepod *Mesocyclops leuckartii* at night (Begg 1976).

Although differential encounter and attack probabilities appear to be the dominant factors determining the feeding selectivity of visual-feeding planktivores (Werner and Hall 1974; Confer and Blades 1975; O'Brien et al. 1976), capture probabilities are still influential. Like our simulated suction, visual-feeding planktivores capture cladocerans with greater success than copepods. The sunfish (*Lepomis gibbosus*) captured *Daphnia* sp. on 100% of its attacks, *Diaptomus sicilis* on 79%, and *D. ashlandi* on 39% (Confer and Blades 1975). The difference between these diaptomid capture probabilities and our *D. pallidus* capture probabilities may be due not only to species differences but also sunfish capture mechanics. A sunfish can increase its capture probability by either positioning its suction closer to the prey or learning to recognize mobile prey and altering its feeding mechanics to increase its intake speed. These differential capture probabilities can alter the planktivore's size-selectivity. The alewife (*Alosa pseudoharengus*) elected *Bosmina*, which are small but easily captured, over cyclopoid copepods, which are large but able to escape (Hutchinson 1971).

Differences in escape abilities between cladocerans and copepods account for differences in their ecology as well as aspects of zooplankton community structure. Copepods can remain higher in the water column than cladocerans (Langford 1953) where encounter and attack by planktivores is most intense. In deep lakes containing visual-feeding planktivores, copepods are often larger than cladocerans (Brooks and Dodson 1965; Hutchinson 1971) because their low capture probabilities can offset high encounter and attack probabilities associated with larger zooplankter size.

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First Evidence for Even-Year Spawning Pink Salmon, *Oncorhynchus gorbuscha*, in Lake Superior[1]

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KWAIN, W., AND J. A. CHAPPEL. 1978. First evidence for even-year spawning pink salmon, *Oncorhynchus gorbuscha*, in Lake Superior. J. Fish. Res. Board Can. 35: 1373–1376.

Nine even-year spawning pink salmon, *Oncorhynchus gorbuscha*, were taken in Steel River, Lake Superior, Ontario, in 1976. The possible origin of these fish was from unknown plantings or spawning of 3-yr-old, odd-year spawning pink salmon.

Key words: Great Lakes, distribution, salmon maturation, spawning age, introduced salmon

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On a capturé 9 saumons roses, *Oncorhynchus gorbuscha*, frayant en année paire dans la rivière Steel, lac Supérieur, Ontario, en 1976. Ces saumons provenaient possiblement de peuplements inconnus ou du frai de saumons roses frayant en année impaire âgés de 3 ans.

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PINK salmon, *Oncorhynchus gorbuscha*, have thrived in Lake Superior after their inadvertent introduction in 1956 (Nunan 1967). Their distribution has expanded and in 1975 spawning pink salmon were found in almost every tributary of the lake; some were also reported in northern Lake Huron (Collins 1975). Throughout 1956–75 Lake Superior pinks retained their normal 2-yr maturation cycle, spawning only in odd-numbered years. However, on September 24, 1976, a mature pink salmon was taken by an angler in the Steel River, a tributary to Lake Superior (48°47′N, 86°53′W), and additional fish were subsequently taken in experimental nets. The angler reported that he had seen several groups of up to 50 salmon. However, the largest number encountered by our staff was 13 on September 29, on a spawning site utilized by odd-year pinks. Two were collected at that time and six more were taken on October 13. This note documents the first appearance of an even-year salmon spawning run.

Results and discussion — Of the nine fish collected in the fall of 1976, all were spent females. Some of them had a few ripe eggs remaining in the body cavity and there is no doubt that they were spawning in the Steel River. Identification was based on anatomical features (Scott and Crossman 1973). Measurements are given in Table 1 and show that the numbers of gill rakers, branchiostegal rays (10–11), and scales on the lateral line (179–218) were within the range of pink salmon.

Ages were determined by the scale method. It could be clearly seen that the scales had only one completed annulus and substantial summer growth, the same as the odd-year pink salmon (Fig. 1). Scale samples were verified by H. T. Bilton of Pacific Biological Station, Nanaimo, B.C., and confirmed to be 2 yr old. Both even- and odd-year pink salmon had approximately the same number of circuli and width of year band, 23.3 ± 2.42 and 0.78 ± 0.10, respectively. The average number of circuli is comparable to that of pinks originating from Alaskan areas (T. Bilton personal communication).

This is the first known record of even-year freshwater pink salmon being caught in the Great Lakes. A 3-yr-old pink salmon of 49.6 cm was caught on October 29, 1976, in the Chocolay River, Michigan, a tributary of Lake Superior, and clearly indicated two completed annuli, plus summer growth (T. Stauffer, Michigan Department of Natural Resources, personal communication). Although the Steel River run was relatively small in comparison to the odd-year pink salmon run, further expansion is probable and nature seems to have implemented Schumacher and Hale's (1962) recommendation that a strain of even-year pink salmon should be introduced into Lake Superior.

The source of the even-year stock is unknown. However, Lake Superior odd-year pink salmon originated in the Skeena River, B.C., and no other stock has been introduced. Anas (1959) reported a pink salmon from the Skeena River in 1956, whose scales showed two completed annuli and substantial 3rd-yr growth. One other 3-yr-old pink has been reported from the Gulf of Alaska by Turner and Bilton (1968). We assume that during the last 20 yr some odd-year fish must also have lived to 3 yr in Lake Superior and spawned in

TABLE 1. Size and meristic counts for even-year pink salmon, *Oncorhynchus gorbuscha*, (all spent females).

	Total length (cm)	Fork length (cm)	Wt (g)	First gill arch rakers	Branchio-stegal rays	Lateral line scales
Sept. 24	48.2	—	—	30	10	200
Sept. 29	45.7	—	—	29	—	192
Sept. 29	45.7	—	—	28	--	200
Oct. 13	48.5	—	—	28	11	218
Oct. 13	—	42.7	644	28	10	—
Oct. 13	—	44.0	728	28	11	193
Oct. 13	45.1	41.5	629	27	10	179
Oct. 13	—	41.0	633	28	10	—
Oct. 13	—	42.4	615	27	10	189

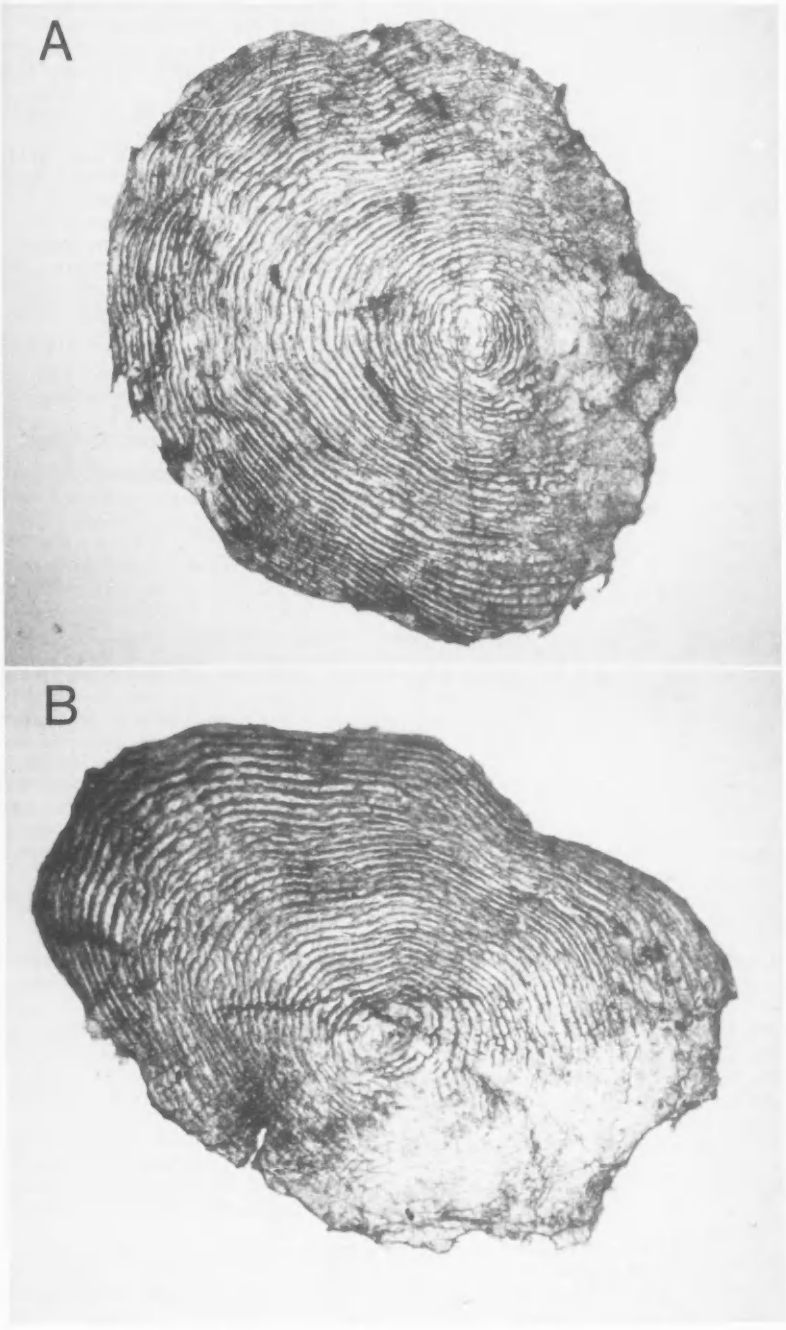

FIG. 1. Scales taken from Lake Superior pink salmon, *Oncorhynchus gorbuscha*. A, Even-year pink salmon caught in Steel River, 1976, 449 mm in fork length; B, odd-year pink salmon caught in Steel River 1975, 380 mm in fork length.

even years to generate the new stock. Scott and Crossman (1973) described pink salmon usually about 61 cm long as marine adults, but usually not over 46 cm in freshwater in eastern Canada. Lake Superior pinks are decidedly slow growing, which may well have favored this late maturity.

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Histopathological and Hematological Changes in Mummichogs, *Fundulus heteroclitus*, Infected by a Pseudomonad[1]

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LI, M. F., G. S. TRAXLER, AND S. CLYBURNE. 1978. Histopathological and hematological changes in mummichogs, *Fundulus heteroclitus*, infected by a pseudomonad. J. Fish. Res. Board Can. 35: 1376–1381.

The hematological and histopathological changes in mummichogs infected by *Pseudomonas reptilivora* included a dramatic reduction in hematocrit values; massive structural lymphoid necrosis with formation of edematous spaces in the splenic tissue; empty and degenerate hepatic veins and sinusoids; and necrosis of the renal tubules in naturally and experimentally infected fish. Electron microscopy of the renal tissue revealed clumps of chromatin in the nuclei, disintegration or degeneration of organelles, and an increase of digestive vesicles or autophagic vacuoles in the cells. This is the first description of the pathology of this bacteria in a marine fish.

Key words: fish disease, *Pseudomonas*, bacteria, hemorrhagic septicemia

LI, M. F., G. S. TRAXLER, AND S. CLYBURNE. 1978. Histopathological and hematological changes in mummichogs, *Fundulus heteroclitus*, infected by a pseudomonad. J. Fish. Res. Board Can. 35: 1376–1381.

Parmi les changements hématologiques et histopathologiques observés chez des choquemorts infectés par *Pseudomonas reptilivora*, on note : une diminution dramatique des valeurs d'hématocrite; une nécrose lymphoïde structurale massive avec formation d'espaces oedémateux dans le tissu de la rate; des veines et sinusoïdes hépatiques vides et dégénérés; et une nécrose des tubules rénaux chez des poissons infectés naturellement et expérimentalement. L'examen au microscope électronique du tissu rénal révèle des agglomérations de chromatine dans les noyaux, une désintégration ou dégénération des organelles et une augmentation des vésicules digestives ou des vacuole autophages dans les cellules. C'est la première description de la pathologie de cette bactérie chez un poisson de mer.

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RED pest or *Vibrio* disease of saltwater eels and fish has been reported frequently (Amlacher 1970; Bullock et al. 1971; Evelyn 1971; Traxler and Li 1972; Reichenbach-Klinke and Landolt 1973), but a similar condition, the hemorrhagic septicemia of marine fish caused by *Pseudomonas* sp., has not been extensively studied. An outbreak of this condition among mummichogs, *Fundulus heteroclitus*, held in our running seawater aquarium (15°C) (Stewart and Power 1962) made this study necessary. Previous work by Li and Traxler (1975) showed that a *Pseudomonas* sp. caused the mass mortalities and could produce typical lesions in experimentally infected mummichogs. The extracellular hemolysin elaborated by the organism appeared to be the agent responsible for the mass mortalities of the infected mummichogs. This report extends the results by describing the hematological and histopathological changes that occurred in the infected fish.

Materials and methods — The mummichogs, approximately 6–8 cm long, were caught near the mouth of Lawrencetown River, N.S., and maintained in our seawater aquarium (15°C) (Stewart and Power 1962). For infection experiments apparently healthy fish were injected intramuscularly or intraperitoneally with 0.05–0.1 mL of washed cells (8×10^7 viable cells/mL) or a 24-h culture (10^7 viable cells/mL). A similar amount of sterile saline or sterile culture medium (brain heart infusion) was injected intramuscularly or intraperitoneally for control fish. Each group of experimental fish was kept in a separate tank with running heated seawater at 15°C. The experimental period was 7 d.

Blood for hematocrit determinations was obtained by severing the caudal vein of the mummichogs and collecting the blood in heparinized microhematocrit tubes. Preparation of the May-Grunwald stained smears from the blood and peritoneal fluid of the experimental fish was described previously (Li 1974).

The tissues from the experimental fish were fixed in Davison solution (Shaw and Battle 1957). The fixed tissues were dehydrated in a series of graded alcohols and embedded in paraffin according to standard procedures. Paraffin blocks were sectioned at 5–8-μm widths and stained with hematoxylin and eosin.

For electron microscopy the cells of the isolated pseudomonad were stained with 1% aqueous potassium phosphotungstate at pH 7.0 (Li and Traxler 1971). The tissues from control or experimentally infected mummichogs were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2–7.3) for 3 h, washed repeatedly with the phosphate buffer, and then fixed in 2% osmium tetroxide in phosphate buffer for 1 h at 4°C. The fixed tissues were dehydrated in a graded series of acetone–water solutions, finishing with 100% acetone, and then embedded in epoxy resin (Durcupan-ACM). Thin sections were stained by Millonig lead and uranyl acetate (Li and Traxler 1972) and examined using a Hitachi-HS9 electron microscope.

Results — Hemorrhagic lesions were observed first on various regions of the body surfaces of the naturally diseased mummichogs. Swollen lesions on the dorsal surfaces and/or muscular spasms in the anal region were seen in the moribund fish. These lesions developed into necrotic areas of ulcerations. Occasionally the body of the diseased fish was filled with a yellowish to blood-colored exudate. Liver, intestine, and the peritoneum became congested.

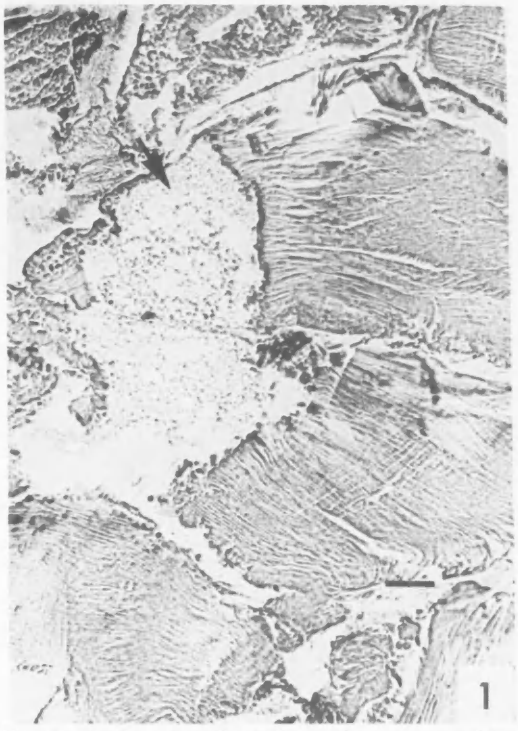

FIG. 1. Section of swollen lesion of diseased mummichog showing degeneration of muscle fibrils (arrow). Hematoxylin and eosin stain. Bar = 50 μm.

The *Pseudomonas* sp. isolated from the diseased fish caused heavy mortalities in experimentally infected mummichogs (Li and Traxler 1975) in which the typical hematological changes and histopathological lesions were reproduced. Figure 1 shows a section of a swollen lesion from a diseased mummichog. Extensive damage was noted in muscle tissue with the muscle fibers apparently undergoing degeneration or outright destruction. The organism was readily reisolated from the experimentally infected fish.

The presence of the pathogenic pseudomonad is shown in the May-Grunwald Giemsa stained preparations of peritoneal fluid and blood of experimentally infected mummichogs (Fig. 2). This organism has lophotrichous flagella and grows readily at 37°C. On the basis of biochemical tests (Li and Traxler 1975), it has been tentatively identified as *Pseudomonas reptilivora*.

A severe anemic condition was observed in most

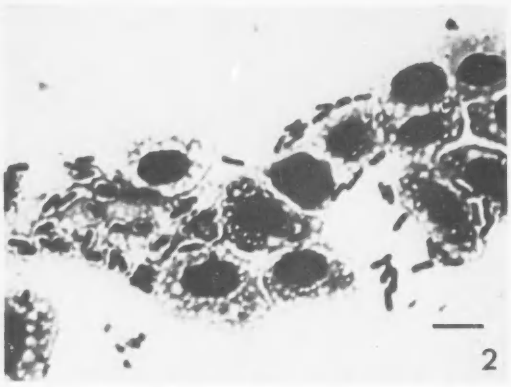

FIG. 2. Smear taken from blood of experimentally infected fish demonstrating massive proliferation of the organism and the degenerating erythrocytes. May-Grunwald and Giemsa stain. Bar = 10 μm.

of the diseased fish. The hematocrit value of the infected blood was significantly lower than that of normal blood (Table 1). A reduction of 55–85% in hematocrit values was observed in the experimentally infected mummichogs when compared with the control fish.

Photographic documentation shows some of the histopathological conditions apparent in the hemopoietic tissues of the infected mummichogs.

The normal spleen showed diffused lymphoid follicles and epitheloid cells while the infected spleen shows massive structural and lymphoid necrosis with formation of edematous spaces. Similar histopathological changes were reproduced in the experimentally infected fish (Fig. 3A, B).

In the infected liver of the fish during natural outbreak, the hepatic cells around the hepatic veins and sinusoids were empty and degenerate. A similar pattern of pathological changes in the liver tissue was observed in the experimentally infected mummichogs. The hemopoietic tissue (HP) and renal tubules (RT) of the experimental control mummichogs are shown in Fig. 4A, and necrotic lesions of the tubules in the infected kidney are observed in Fig. 4B. Electron microscopy of the renal tissues revealed that the cells in the experimental control tissue had evenly diffused chromatin materials in the nucleus and well-developed cytoplasmic organelles (Fig. 5). In contrast, the chromatin of nuclei were severely clumped and organelles had partially disintegrated in the infected cells; an increase of digestive vesicles or autophagic vacuoles was observed (Fig. 6A, B).

Discussion — *Pseudomonas reptilivora* was first iso-

TABLE 1. Comparison of mortality and hematocrit values of normal mummichogs, *Fundulus heteroclitus*, with those infected with *Pseudomonas reptilivora*.

Mummichogs	No. of fish	Route of injection[a]	Injected material[b]	No. dead	Hematocrit mean (range)[c]	Remarks
Normal	8				35 (25–43)	Recently caught near the mouth of Lawrencetown river.
Apparently healthy	8				33.8 (22–41)	During natural outbreak of the disease, over 50% mortality.
Moribund	8				25.5 (13–38)	
Control	12	IM	Sterile seawater	1		One fish died on 7th d, no typical lesion.
Infected (A)	12	IM	Washed cells 0.05 mL/fish	11		7 dead within 48 h after injection.
Infected (B)	15	IP	Washed cells 0.05mL/fish	14		8 dead within 48 h after injection.
Control	10	IP	Sterile medium (BHI) 0.1 mL/fish	0	30.2 (27–35)	No mortality in 7-d experimental period.
Infected (A)	10	IM	24-h culture (0.2 mL/fish)	8	4.7 (3–6)	8 dead within 72 h with lesions; 2 moribund with hemorrhagic lesions.
Infected (B)	10	IP	24-h culture	7	13.8 (8–20)	7 dead within 72 h with lesions; 3 survived, but very inactive with hemorrhagic lesions.

[a]IP, intraperitoneal injection; IM, intramuscular injection.

[b]BHI, brain heart infusion used as culture medium; 24-h culture contained 10^7 viable cells/mL; washed cells, cells repeatedly washed in sterile seawater, contained 8×10^7 cells/mL.

[c]Dead fish were not included for hematocrit value determination. For experimental infection studies hematocrit value was determined from two surviving fish for each group at end of 7-d experimental period. At least two determinations were conducted for each specimen.

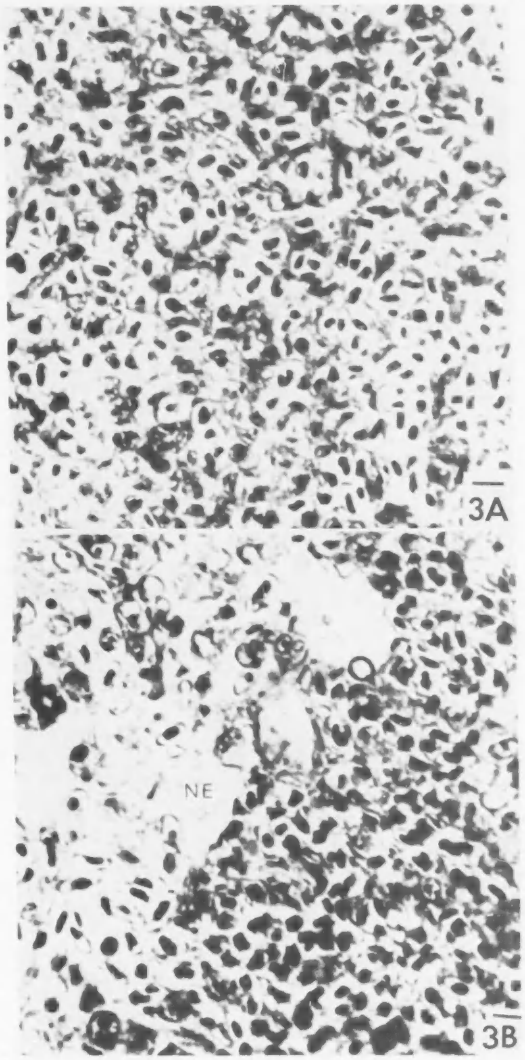

FIG. 3. Splenic tissues from control and experimentally infected mummichogs. A, Normal spleen with diffused lymphoid follicles and epitheloid cells of the control mummichogs; B, infected spleen. Note necrotic lesions (NE). Bar = 10 μm.

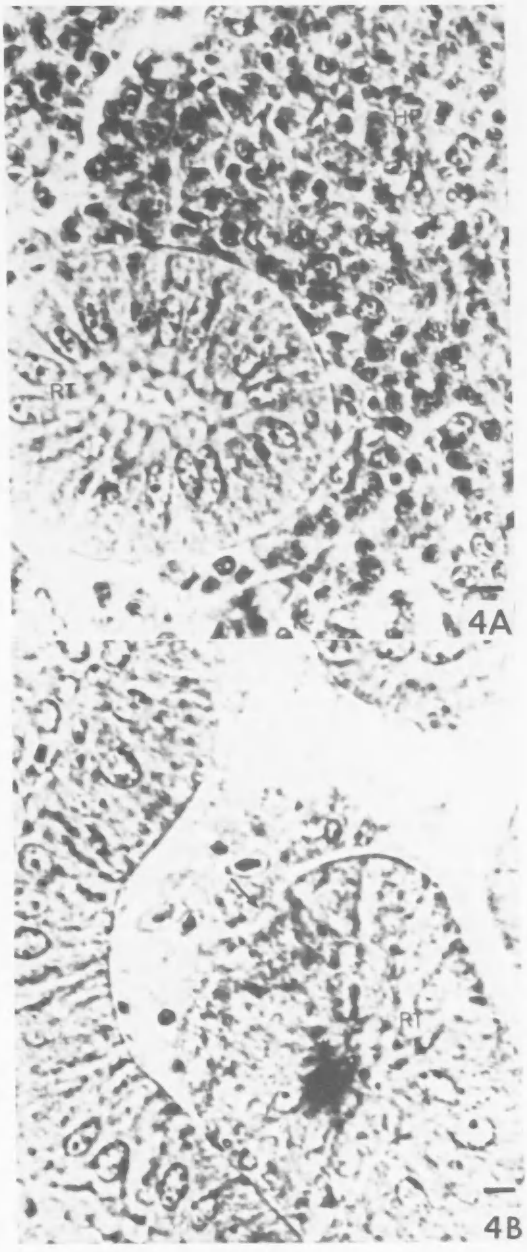

FIG. 4. Kidney tissues from control and experimentally infected mummichogs. A, Normal kidney with hemopoietic tissue and renal tubule; B, cross section of renal tubules of the infected kidney. Note necrotic lesion (arrow). HP, hemopoietic tissue; RT, renal tubules. Bar = 10 μm.

lated from diseased reptiles by Caldwell and Ryerson (1940) and is highly pathogenic for the lizard *Phrynosoma solare*, Gila monsters (*Heloderma suspectum*), and Chuckasalla (*Sauromalus ater*). No citations of *P. reptilivora* as the causative agent for any fish disease have been found in our literature searches.

The extracellular proteinase of other pseudomonads pathogenic to fish was investigated earlier (Li and Flemming 1967; Li and Jordan 1968). This organism produces both powerful proteinase and hemolysin (Li and Traxler 1975). The massive muscular destruction

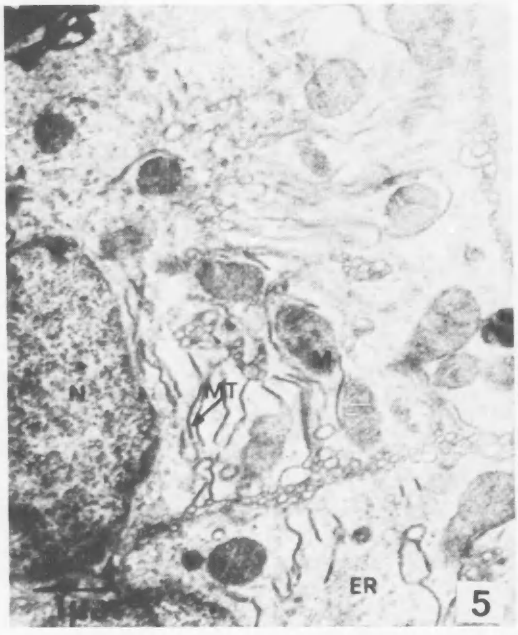

FIG. 5. Electron micrograph of control kidney cells. Note evenly diffused nuclear chromatin, well-developed mitochondia, smooth reticulum, and numerous cytoplasmic microtubules. N, nucleus; M, mitochondia; ER, smooth reticulum endoplasm; MT, microtubules. Bar = 1 μm.

and dramatic reduction of hematocrit value of the infected fish apparently resulted from the proteinase and hemolysin elaborated by the organism (Table 1).

Hematocrit values for fish, however, vary with the environmental conditions as well as with the nutritional and physiological conditions of fish (Blaxhall 1972). In addition, the hematocrit values for the experimentally infected mummichogs were determined at the end of the 7-d experimental period. These could partially account for the fact that the hematocrit values were lower in the experimentally infected fish than in the naturally infected fish (Table 1).

The hemorrhagic septicemic condition of the diseased fish is further illustrated by cellular degeneration, necrosis and edematous formation in hematopoietic elements, and lack of erythrocytes in the hepatic sinusoids and veins. The marked clumping of nuclear chromatin and increased formation of digestive vacuoles are common cellular reactions to injuries. Furthermore, the disintegration or decreased regeneration of cytoplasmic organelles could result from the damage by bacterial toxins (Trump and Arstila 1975). The strong hemolytic and proteolytic activities of the isolated pseudomonad, the hemorrhagic lesions, severe septicemic and anemic conditions in conjunction with cytological evidence constitute a typical syndrome of hemorrhagic septicemia for this bacterial disease. These

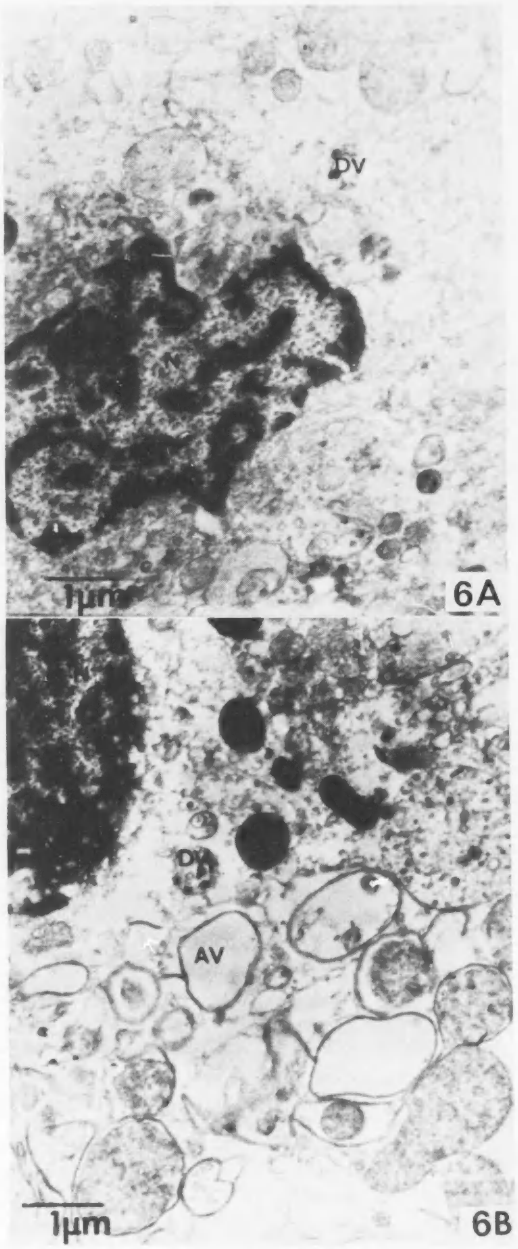

FIG. 6. Electron micrographs of kidney cells from the infected renal tissue. Note (A) severely clumped nuclear chromatin and disintegrated cytoplasm and (B) numerous digestive vesicles and autophagic vacuoles. N, nucleus; DV, digestive vesicles; AV, autophagic vacuoles. Bar = 1 μm.

observed histopathological and hematological changes in mummichogs will form the basis for future pathology

studies in other marine fishes infected by this pseudomonad.

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Status of *Exosphaeroma inornata* Dow and *E. media* George and Stromberg (Isopoda: Sphaeromatidae) with Ecological Notes

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IVERSON, E. W. 1978. Status of *Exosphaeroma inornata* Dow and *E. media* George and Stromberg (Isopoda: Sphaeromatidae) with ecological notes. J. Fish. Res. Board Can. 35: 1381–1384.

The sphaeromatid isopod *Exosphaeroma media* George and Stromberg 1968 from San Juan Island, Puget Sound, is synonymized with *E. inornata* Dow 1958 from California. Differences between the original descriptions of both species are attributed to author errors or variations in the phenotype. Variation in the relative position of the rostrum and clypeus, crenulation of the uropod rami, pleotelson shape, coxal plate suture lines, and body ratio (width/length) are discussed. *Exosphaeroma inornata* is reported to be a wide-ranging intertidal species which is commonly found in crevices, sand, and associated with various algae. Although the distribution is reported to be from San Diego, Calif., north to San Juan Island, Puget Sound, a large distribution gap exists between Humboldt Bay and Puget Sound.

Key words: isopod synonomy, anatomical variations, distribution, habitat

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IVERSON, E. W. 1978. Status of *Exosphaeroma inornata* Dow and *E. media* George and Stromberg (Isopoda: Sphaeromatidae) with ecological notes. J. Fish. Res. Board Can. 35: 1381–1384.

L'auteur démontre que l'isopode sphaeromatide *Exosphaeroma media* George et Stromberg 1968 en provenance de l'île San Juan, Puget Sound, est synonyme de *E. inornata* Dow 1958 provenant de la Californie. Il attribue à des erreurs de la part des auteurs ou à des variations de phénotypes les différences dans les descriptions originelles des deux espèces. Il analyse la variation de la position relative du rostre et du clypeus, la crénulation des rami de l'uropode, la forme du pléotelson, les lignes de suture des plaques coxales et le rapport corporel (largeur/longueur). *Exosphaeroma inornata* est une espèce intertidale dont l'aire de distribution est étendue et qui se rencontre ordinairement dans les crevasses, le sable, et associée à diverses algues. Bien qu'on ait mentionné l'aire de distribution comme s'étendant de San Diego, Californie, à l'île San Juan, Puget Sound, au nord, il existe un vide important entre la baie Humboldt et le Puget Sound.

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THE sphaeromatid isopod *Exosphaeroma media* George and Stromberg 1968 was described from the upper intertidal zone of San Juan Island, San Juan Archipelago, Puget Sound. However, due to an unfortunate error in figure 8A of their description, *E. media* appears to differ from all other genera of Sphaeromatidae. They figure the fourth pleonite as being free, not medially fused to the two anterior pleonites. If George and Stromberg's interpretation of the fourth pleonite structure is disregarded, *E. media* then shows a close resemblance to *E. inornata* Dow 1958, known from central and southern California.

The general resemblance between *E. media* and *E. inornata*, the apparent discrepancy in the description of the pleon in the former species, and the occurrence of both species in similar habitats in Puget Sound, Washington (see below) suggested that these species might be synonymous. A comparison of the type material confirmed this suspicion.

Materials examined — The supplemental descriptive notes given below are based on the paratypes of *E. media* (USNM 119837) and the type series of *E. inornata* (Holotype, Los Angeles County Museum 1113; Allotype, LACM 1114; and 232 paratypes) plus a large series of males, females, and juvenile stages from six California populations (Point Fermin, Los Angeles County; Carpenteria State Beach, Santa Barbara County; Half Moon Bay, San Mateo County; San Francisco Bay, San Francisco County; Bodega Head, Marin County; and Humboldt Bay, Humboldt County) and one population from Puget Sound, Washington, (Smith Cove, Seattle). Additional distributional records were obtained from materials deposited in the California Academy of Sciences collections (CAS), and specimens collected by the University of California, Irvine, for the Bureau of Land Management (BLM).

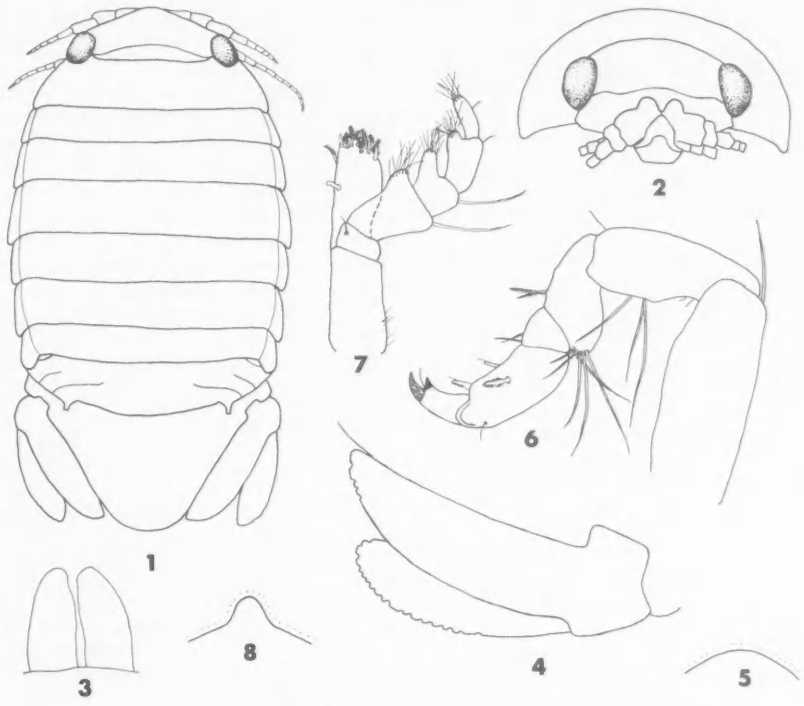
Supplemental descriptive notes — The original descriptions by Dow (1958) and George and Strombert (1968) give several characters that might serve to separate *E. inornata* from *E. media*. However, reexamination of the characters showed that these differences were due to either author errors or to variations in the phenotype. Except where refigured here, the drawings given by Dow (1968) are considered to adequately represent *E. inornata* and are not duplicated.

George and Stromberg's figure 8A incorrectly shows the pleon with the fourth pleonite entirely free, not medially fused with the anterior two segments. In the text they describe the pleon as "Anterior segment with two suture lines; half as long as telson." In the genus *Exosphaeroma*, the first pleonite lies under the seventh pereonite, obscuring it from dorsal view except near the articulation of the seventh pereonite and its coxal plate (Fig. 1). The second, third, and fourth pleonites fuse medially into the second pleonal division, forming the dorsally visible part of the pleon. The fifth and sixth pleonites fuse with the telson forming a pleotelson. A reexamination of George and Stromberg's paratypes clearly showed the fourth pleonite is medially fused to the two anterior pleonites.

The anterior margin of the cephalon as described by George and Stromberg is correct. In addition, the supraantennal line is poorly developed, and weakly trilobate. The relative position of the rostrum to the clypeus is variable. In some individuals the rostrum clearly touches the clypeus, while in others the rostrum and clypeus are widely separated. Typically the rostrum and clypeus are nearly touching (Fig. 2).

The incisor process of the mandible ends in four rounded cusps. Dow incorrectly describes and figures the lacina mobilis terminating in two instead of three cusps. George and Stromberg's figure 8J incorrectly shows the paired peni as medially fused over the proximal fourth of its length. The peni are clearly separated at the proximal margin (Fig. 3). George and Stromberg's figure 8I incorrectly shows the appendix maxculina of the male second pleopod as biarticulate. The appendix masculina actually is unsegmented, attaching to the medial proximal margin of the endopod.

George and Stromberg correctly stated that the border of the uropodal rami have slight emarginations which give them a crenulated appearance (Fig. 4). Some variation was observed in this character by George and Stromberg (1968). In this study, several



FIGURES 1–8. *Exosphaeroma inornata*

Fig. 1. Adult female. Fig. 2. Cephalon. Fig. 3. Male, peni. Fig. 4. Uropod. Fig. 5. Juvenile, posterosaggital view of pleotelson apex. Fig. 6. Female, pereopod one. Fig. 7. Maxilliped. *E. amplicauda.* Fig. 8. Juvenile, posterosaggital view of pleotelson apex.

individuals that completely lacked crenulations on both rami were found (agreeing with Dow's description), while in extreme cases the distal and lateral margins of both the exopod and the endopod are crenulated. Typically only the distolateral margin of the exopod is crenulated. The shape of the pleotelson varies slightly with age and sex. Females and immature males have a broadly rounded posterior margin which becomes increasingly truncate in large adult males. Suture lines of coxal plates two to seven with their respective pereonites are distinguishable (best seen in lateral view) on females and juvenile males. Coxa and pereonites appear completely fused in adult males.

Schultz (1968) distinguishes in his key between *E. inornata* and *E. media* by the more convex body margins of the latter species. Computation of a body ratio (width/length) from the respective measurements of length and width given by Dow (holotype = 0.27, allotype = 0.26) and George and Stromberg (holotype = 0.50, allotype = 0.50) for their types agrees with Schultz's observations. However, remeasurement of Dow's paratypes revealed the body ratio ranged between 0.46 and 0.53, suggesting that his measurements of the length and width of the holotype and allotype are in error. Precise measurements can no longer be made from these specimens since they have suffered from decalcification. Freshly collected topotypes of *E. inornata* from Point Fermin, Calif., averaged 0.54. This value falls well within the range of body ratio measurements recorded from 127 individuals (0.44 to 0.59, mean = 0.55).

The setation of the first pereopod (Fig. 6) was found to vary a great deal more than the variation reported by George and Stromberg. The number of setae on the outer margin of the ischnum ranges from 3 to 12 long setae; distally, the meris ranges from 4 to 13 setae; the inner margin of the carpus ranges from one to three short setae; and the inner margin of the propodus ranges from two to five setae. Typically, the inner margin of the propodus has two, two-pointed setae plus a stout plumose setae on the lateral margin, as per George and Stromberg's description, but contra to Dow's figure. Figures given by both Dow and by George and Stromberg of the maxilliped are within the range of variation observed. Figure 7 represents a maxilliped of average proportions.

In most cases juvenile sphaeromatid isopods are difficult to identify because distinguishing characters pres-

ent in the adult are often reduced or absent. Small juvenile specimens of *E. amplicauda* lack the characteristic dorsal sculpturing and angulate pleotelson apex of the adult, thus resembling *E. inornata*. The latter species can be distinguished from the former species by the absence of a groove or channel on the ventral surface of the pleotelson apex, (Fig. 5 and 8). As juvenile *E. amplicauda* mature, the pelotelson apex becomes increasingly more angulate, while in *E. inornata* it remains broadly rounded.

Ecology — George and Stromberg (1968) collected their specimens from several localities on San Juan Island, in the upper intertidal zone from ". . . clean sand and among stones in slight depressions of the beach, where coarser material accumulates." I collected a large series of *E. inornata* at Smith Cove, Seattle, Washington (about 100 km south of San Juan Island) in August 1973. Here, *E. inornata* occurs in the middle and upper intertidal zones among the stones of a cobble stone beach, associated with the alga *Ulva* sp., and in the empty tests of the barnacle *Balanus glandula* Darwin 1854. In California *E. inornata* is widely distributed in many habitats including the fully exposed rocky outer coast and the fully protected marine regions of bays and harbors. It is not known from the sandy outer coast beaches. At Bodega Head, Calif., *E. inornata* was found in coarse sand, in between individuals of the clone-forming anemone *Anthopleura elegantissima* (Brandt 1835).

Dow (1958) stated that *E. inornata* occurs under the holdfast of the kelp *Macrocystis*. In southern California, the dominant species of *Macrocyctis* is *M. pyrifera* (Linnaeus 1771). This kelp forms extensive stands offshore at depths commonly between 6 and 20 m (Abbott and Hollenberg 1976). Dow's statement thus implies that *E. inornata* occurs subtidally between those depths. Dr J. L. Mohr collected the material described by Dow (1958) at Point Fermin, Los Angeles County, Calif., in 1948. Dr Mohr has informed me (personal communication) that in 1948 he did not have any means to reach the offshore kelp stands at Point Fermin. Thus, the material described by Dow probably came from holdfasts that washed up on the beach. Once in the intertidal, the holdfast could easily have been colonized by the isopod . Although numerous museum records have been examined, as yet no subtidal records are known to me. Thus, *E. inornata* is probably strictly a littoral species.

Distribution — Previous to this report, the known distribution of *E. inornata* was from Point Fermin, Los Angeles County, Calif. (type-locality) north to Humboldt Bay, Humboldt County, Calif. (Iverson 1974). The distribution is now extended north to San Juan Island, Puget Sound (George and Stromberg 1968) and south to San Diego, San Diego County, Calif. (BLM data). Numerous intermediate localities are known within its reported range in California. A large distributional gap exists between Humboldt Bay and Puget Sound, probably reflecting the lack of adequate sampling in this area. In California, *E. inornata* is also known from three offshore islands: South East Farallon Island, off San Francisco Bay (CAS data); San Nicolas Island (BLM data); and San Miguel Island (BLM data).

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Mating Behavior of the Rock Crab, *Cancer irroratus*

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ELNER, R. W., AND A. B. STASKO. 1978. Mating behavior of the rock crab, *Cancer irroratus*. J. Fish. Res. Board Can. 35: 1385–1388.

A pair of rock crabs, *Cancer irroratus*, in mating embrace an hour after being placed in a laboratory tank, was observed intermittently until ecdysis of the female and copulation of the pair, and then until escape of the female 27 d after ecdysis. When separated from the mating embrace, the hard-shelled female sought out the male. Approach of female ecdysis was indicated by protrusion of a soft spherical bulge at the rear of the epimeral line. Details of the immediate pre- and postcopulatory behavior were observed continuously from 26 min before to 150 min after copulation. The female was first noted separated from the male 7 h after copulation. Periods of separation increased until mating interactions were completely abandoned 5 d after copulation. A second pair subsequently observed confirmed the above. Mating embrace was also observed between a hard-shelled male and a hard-shelled female that already had sperm plugs.

Key words: Crustacea, crabs, copulation, molt indicators

ELNER, R. W., AND A. B. STASKO. 1978. Mating behavior of the rock crab, *Cancer irroratus*. J. Fish. Res. Board Can. 35: 1385–1388.

Un couple de crabes-tourteaux communs, *Cancer irroratus*, s'accouplèrent une heure après avoir été placés dans un vivier en laboratoire. Nous les avons observés de façon intermittente jusqu'à la mue de la femelle et la copulation du couple, et ensuite jusqu'à la fuite de la femelle 27 jours après la mue. Une fois séparée de l'étreinte nuptiale, la femelle à carapace dure rechercha le mâle. L'approche de la mue chez la femelle fut signalée par l'apparition d'un renflement sphédique et mou à l'arrière de la ligne épimérale. Les détails du comportement immédiatement avant et après copulation ont été observés de façon continue de 25 min avant à 150 min après copulation. On a vu pour la première fois la femelle séparée du mâle 7 h après la copulation. Les périodes de séparation se firent de plus en plus longues, jusqu'à ce que les interactions d'accouplement furent complètement abandonnées 5 jours après copulation. Ces observations ont été corroborées par un second couple observé par la suite. On a également observé l'étreinte nuptiale d'un mâle et d'une femelle, tous deux à carapace dure, cette dernière ayant déjà des spermatophores.

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THE rock crab, *Cancer irroratus*, is found along the Atlantic Coast of North America from South Carolina to Labrador (Rathbun 1930; Squires 1966). In the southern part of its range it lives offshore to 400 m depth, migrating inshore during the winter months (Haefner 1976; Shotton 1973). In Canadian waters it lives close to shore and is particularly abundant in the southern Gulf of St. Lawrence (Stasko 1976). At present it is of only limited commercial value over most of its range, due largely to the high costs of processing. The life cycle of the rock crab in southern waters is described by Haefner (1976) and Haefner and Van Engel (1975); and in northern waters by Scarratt and Lowe (1972) and Krouse (1972, 1976).

The mating behavior of other *Cancer* crabs has been described by Edwards (1966, *Cancer pagurus*) and Snow and Neilson (1966, *Cancer magister*), and resembles that of the blue crab, *Callinectes sapidus* (Chidester 1911). Mating behavior of the rock crab, *Cancer irroratus*, according to Chidester (1911) is also similar to that of the blue crab. Although he mentions some unusual mating interactions of rock crabs, Chidester did not describe their normal mating and copulatory behavior. Brief mention of rock crab copulation is made by Terretta (1973).

In the present paper we describe the behavior before copulation, the act of copulation, and postcopulatory behavior of *Cancer irroratus*.

Materials and methods — Several dozen rock crabs were captured by otter trawl on October 4, 1977, at a depth of about 12 m in Passamaquoddy Bay, New Brunswick, near the mouth of the Bay of Fundy. Bottom water temperature was 11.3°C. An hour after the crabs were placed in 90 × 90 cm laboratory tanks with 20 cm of sea water, a pair was observed in mating embrace. The pair was transferred to a

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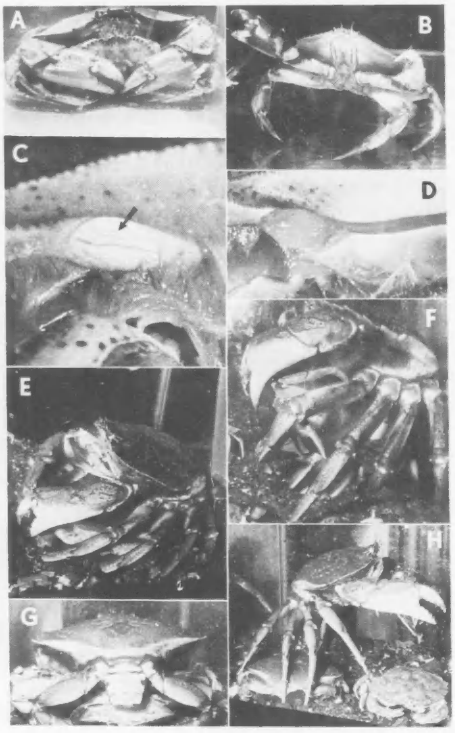

FIG. 1. (A) A large male (121 mm) and small female (62 mm) rock crab (*Cancer irroratus*) in the mating embrace; (B) male assuming a typical defensive meral spread; (C) normal condition of bulge (arrowed) at the posterior end of the pleural line in females during the intermolt period; (D) more distinct pleural line and swollen bulge of a female close to ecdysis; (E) male in a slightly elevated position above soft-shelled female soon after ecdysis and before copulation; (F) male in elevated position above female who is in the process of turning over and clinging to the male's underside just prior to copulation; (G) position shortly after copulation with upside-down female on substrate and male crouched over her with extended abdomen; (H) male in extreme elevated position over righted female about 95 min after copulation. Note discarded empty shell of female in right foreground.

50 × 35 cm glass aquarium with 25 cm of running seawater at 12°C and kept under intermittent observation. Details of the copulatory behavior were recorded with camera and tape recorder. Size of the shell, measured across the widest part of the carapace from tip to tip of the most distal marginal teeth, was 121 mm for the male and 62 mm for the female. Males reach a maximum carapace width of 140 mm; females reach 105 mm.

Subsequent to the above observations, a pair of crabs (male 130 mm, female 60 mm) collected from the same location on March 3, 1978, was observed through the mating process. The mating of this pair is not described in detail, but, except for the normalization of epimeral bulges at ecdysis, the behavior closely resembled that of the first pair.

Results — Mating embrace — In the mating embrace the male clasped the female, sternum to sternum, with his chelipeds around her carapace. The anterior of the female faced in the same direction as the male and her legs were tucked close to her body (Fig. 1A). The position of the female's carapace varied at times from vertical to almost horizontal. While in the mating embrace the male could walk around carrying the motionless female. Only rarely did the female struggle, by extending her legs and chelipeds as if pushing herself away from the male. At such times the male used his chelipeds and first pair of walking legs to manoeuver the female back into position.

Whenever the pair was forcibly separated, the female approached the male who spread his legs and helped to position the female under himself using his chelipeds and first pair of walking legs. The female cooperated actively and the mating embrace was resumed usually within 30 s. When another male crab was introduced, the mating male extended his chelipeds in a threatening meral spread towards the intruder, while still cradling the female between his cheliped coxopodites. Similar defensive behavior (Fig. 1B) was enacted by unattached males towards observers.

Changes in epimeral line — An external morphological indicator of approaching molt in the female rock crab was the appearance of the epimeral line (subbranchial ecdysial suture line) along the posterior sides of the carapace (Haefner and Van Engel 1975). The line became increasingly distinct and wider as ecdysis approached. At the posterior end of the epimeral line next to the abdomen, hard-shell intermolt females normally have a hard white bulge (Fig. 1C). Such a bulge is not present in males. At the time of capture, ecdysis minus 4½ d, the mating female's bulge was translucent and about 4.2 mm diam (Fig. 1D). By ecdysis minus 3 d the bulge had enlarged becoming spherical and 5.0 mm across. The bulge increased to 5.3 mm on the day before ecdysis.

After ecdysis, the bulge was still present with a diameter of 4.2 mm. Over the next 27 d, until the female escaped, the bulge remained at the same size but became harder and more opaque. In the second pair the bulge reduced to normal intermolt proportions and opacity within 3 h of ecdysis.

Carapace width of the two molting females increased from 62 to 76 mm (22.6%) and from 60 to 72 mm (20%), respectively.

Sequence of mating events — The female molted on October 9 sometime between observations at 09:15 and 11:15, 4½ d after arrival in the laboratory. At 09:15 there were no behavioral signs to indicate ecdysis was imminent. By 11:15 ecdysis had occurred and the soft-shelled female was resting motionless on the substrate,

legs tucked under, with the male in a slightly elevated position above her (Fig. 1E).

Neither animal moved for 25 min. At 11:40 the male raised himself higher off the substrate and prodded the female with his chela. The female rotated about 90°, raised her anterior, and turned over on her back as if walking up the ventral side of the male (Fig. 1F) to form a mating embrace. At this point the male started rocking back and forth, on the tips of his walking legs, at about 15 times/min. Meanwhile the female extended her abdomen nearly straight out while the male extended his only partially so that the tip of his abdomen was folded under that of the female. Actual copulation presumably took place at this point, at 11:41, and lasted approximately 15 s, after which the female folded her abdomen and the male eased his embrace allowing the female to drop to the substrate on her carapace. The male crouched above the upside-down female for nearly 40 min (Fig. 1G), flexing his abdomen about once every 4 s and periodically extending his mouthparts; the female remained motionless. At 12:20 the female, unaided by the male, took up the sternum to sternum mating embrace underneath the male for a further 55 min as the male continued to extend and flex his abdomen but at a decreasing frequency. At 13:15 the female was released from the embrace and turned over unaided to a normal upright position, flat on the substrate, legs tucked under. The male assumed an extreme high stance above the female (Fig. 1H). This stance was gradually relaxed after about an hour.

During the next 24 h the male tended to remain crouched over the female forming a protective cage with his legs and chelipeds (as in Fig. 1E). When disturbed, the male reassumed the extreme high stance extending his claws in the defensive meral spread. At 19:25, nearly 7 h after copulation, the female was observed away from the male for the first time. The periods of separation gradually increased, but when disturbed, the female immediately sought shelter beneath the male. By 5 d after copulation the female had ceased all efforts to seek cover under the male.

During the pre- and immediate postcopulatory period both animals refused food. Food was first accepted by both male and female 2 d after copulation.

When first examined 2 d after copulation the female had two sperm plugs that were not present before copulation. Eggs had not been extruded 27 d after copulation when the female escaped from the aquarium and died. The female in the second pair, collected in March, had not extruded eggs 32 d after copulation.

Another pair of crabs in a mating embrace, collected at the same time as the first pair, was isolated in a separate tank. Initially, the pair was in the mating embrace continuously, but over a 3-d period they were observed to be separated progressively more often. After 3 d in the laboratory they were not seen in the mating embrace again. The female had a completely hard shell, with hard bulges at the rear of the epimeral lines (as in Fig. 1C) and two sperm plugs.

When this other pair was disturbed either by the observer, or by another male crab, the male extended his chelipeds in a defensive meral spread. When the mating pair was separated then put back into the tank, the male moved to the edge, his back against the wall. The female was the one who made the approach. When the original female was replaced with a different hard-shell female, who did not make an approach after several minutes, the male would move towards the female and attempt unsuccessfully to manoeuver her into the mating embrace.

Discussion — The general behavioral pattern and duration of mating events observed for the rock crab are similar to those described by Watson (1972) for the snow crab, *Chionoecetes opilio*, Chidester (1911) for the blue crab, *Callinectes sapidus*, and for other Cancridae (Edwards 1966; Hartnoll 1969; Snow and Neilsen 1966).

The mating embrace of *Cancer irroratus* resembles *C. magister* (Snow and Neilsen 1966), and *C. productus* (C. D. Snow personal communication), in that the female is carried underneath the male sternum to sternum. In other Cancridae described (*C. gracilis*, *C. oregonensis*, *C. pagurus*, *C. productus*) the female is carried with her dorsal carapace against the male's sternum (Hartnoll 1969).

Ecdysis of the female rock crab during the mating sequence was not observed but can be expected to be similar to that described by Snow and Neilsen (1966) for the Dungeness crab, *C. magister*.

Copulatory and postcopulatory behavior similar to that of the rock crab has been described for other crab species, including the Cancridae (Hartnoll 1969). Flexing of the male's abdomen after copulation has hitherto been unreported for the Cancridae, although Watson (1972) detailed similar behavior in the snow crab, and C. D. Snow (personal communication) has observed it in *C. magister* both before and after copulation.

Such mating behavior, with the female protected by the male during her molt, has considerable survival value. Crustaceans are particularly vulnerable to predation while molting and in the soft-shelled state, and normally seek shelter during this period. By forming a mating embrace with a hard-shelled male the female improves her chances of surviving the vulnerable molting period and copulating while her shell is still soft.

The significance of the mating embrace between a male rock crab and a hard-shelled, already-mated female is obscure. Chidester (1911) replaced soft-shelled females in two mating rock crab pairs with hard-shelled ovigerous females, in which spermatophore plugs were not protruding. In both cases he reports that actual copulation occurred. Hartnoll (1969) observed mating hard-shelled females of the families Grapsidae, Ocypodidae, Majidae, and Xanthidae.

At the north end of Northumberland Strait mating

pairs of rock crabs have been noted from July to October (Scarratt and Lowe 1972) while soft-shelled females were most abundant in May and June, with a smaller peak in September and October (A. B. Stasko and C. Stewart unpublished data). In Chesapeake Bay, which is near the southern end of the range of *C. irroratus*, molting of females does not occur until late December (Terretta 1973). Egg extrusion in Northumberland Strait commences in late October (A. B. Stasko and C. Stewart unpublished data). For the two rock crab pairs observed in this study, egg extrusion had not occurred 27 and 32 d after copulation. By contrast, egg extrusion in snow crabs, *Chionoecetes opilio*, takes place between $3\frac{1}{2}$ and 72 h after copulation (Watson 1972). Krouse (1972) and Scarratt and Lowe (1972) suggest that female rock crabs mature at 55–60 mm carapace width and males at 70 mm. This makes it reasonable to assume that the observed copulating pairs were mature animals. Indeed, Rhode Island samples have revealed egg-bearing rock crabs as small as 14 mm, with many collected in the 14–25 mm carapace width range (Reilly 1975).

The state of the epimeral line and protruding bulges of the female rock crab, when properly quantified, could possibly provide an accurate indication of the time interval remaining to ecdysis.

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Effect of Seasonal Photoperiod on Acute Toxic Responses of Juvenile Rainbow Trout (*Salmo gairdneri*) to Pulpmill Effluent

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MCLEAY, D. J., AND M. R. GORDON. 1978. Effect of seasonal photoperiod on acute toxic responses of juvenile rainbow trout (*Salmo gairdneri*) to pulpmill effluent. J. Fish. Res. Board Can. 35: 1388–1392.

Acute toxic responses to bleached kraft pulpmill effluent were compared for groups of underyearling rainbow trout (*Salmo gairdneri*) reared under constant temperature and either a summer (16L:8D) or winter (8L:16D) photoperiod. Toxicity tests included a

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96-h LC50 bioassay, a 4.5-h sealed-jar test, an 18-h temperature tolerance test, and a 4-h blood-sugar stress bioassay. For all tests the threshold-effect concentration of effluent was not affected by photoperiod. Median effective concentrations for the blood-sugar, temperature tolerance, and sealed-jar tests were, respectively, <0.2, 0.4, and ~ 1.4 times the 96-h LC50 value for each fish group.

Key words: Salmo gairdneri, photoperiod, BKME, acute toxicity tests, LC50, blood sugar, temperature tolerance, residual oxygen

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Nous comparons les réponses de toxicité aiguë aux eaux usées de blanchiment d'une papeterie kraft de groupes de truites arc-en-ciel (*Salmo gairdneri*) de moins d'un an élevées à une température constante et avec photopériode d'été (16j:8n) ou d'hiver (8j:16n). Les essais de toxicité inclurent un essai biologique de CL50 après 96 h, un essai de 4.5 h en bocal scellé, un essai de tolérance de température de 18 h et un essai biologique de stress par sucre sanguin de 4 h. Dans tous ces essais, les concentrations de seuil des eaux usées ne sont pas affectées par la photopériode. Les concentrations effectives médianes pour le sucre sanguin, la tolérance de température et les essais en bocal scellé sont respectivement < 0.2, 0.4 et ~ 1.4 fois la valeur de la CL50 après 96 h pour chaque groupe de poissons.

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A number of rapid toxicity tests have been developed in our laboratory as candidate procedures for the routine monitoring of the threshold concentrations of pulpmill effluents which are harmful to salmonid fish. Standardization of these or alternative procedures for application throughout the year requires knowledge of the influence of seasonal variables such as photoperiod and temperature on the tests' sensitivity. In this regard the present study was undertaken to determine the effect of photoperiod on the response of underyearling rainbow trout (*Salmo gairdneri*) to pulpmill effluent according to the conventional 96-h LC50 (median lethal concentration) bioassay as well as a sealed-jar bioassay at ambient room temperature (McLeay 1976; Gordon and McLeay 1977), a temperature tolerance test (McLeay and Howard 1977), and a blood-sugar stress bioassay (McLeay 1977). Test responses for two groups of fish acclimated to identical conditions other than photoperiod (16L:8D "summer" versus 8L:16D "winter" conditions) were compared using a single sample of bleached kraft whole mill effluent.

Materials and methods — Rainbow trout, obtained as swimup fry from Troutlodge Inc. (Washington State), were held in a fiberglass rearing trough at 11.5 ± 0.5°C under "natural" photoperiod conditions (overhead incandescent lighting regulated by photocell) for 1 mo. Subsequently, a group of 2500 fish was transferred to each of two 1000-L fiberglass tanks, housed within a partitioned, controlled environment room. Overhead incandescent illumination (four 100-W bulbs) to each tank was regulated by timer and automated rheostat (30-min dawn/dusk interval) to provide either a summer (16L:8D; light onset 04:30) or winter (8L:16D; light onset 08:30) photoperiod. Light intensity at the water surface of each tank was 450 lx. Fish-rearing conditions other than day length were identical. Water supply for tanks (12–15 $L \cdot min^{-1} \cdot tank^{-1}$) and bioassays was dechlorinated Vancouver City tap water (11.3 ± 0.7°C, pH 6.5 ± 0.2 SD, conductance 15.4 ± 1.5 $\mu mho \cdot cm^{-1}$, EDTA hardness 5.7 ± 0.7 mg $CaCO_3 \cdot L^{-1}$, and alkalinity 5.0 ± 0.8 mg $CaCO_3 \cdot L^{-1}$). Fish were fed Oregon Moist Pellets with the initial ration offered 4 h after the onset of each respective light cycle. Feeding was withheld for 24 h before the initiation of the toxicity tests.

After an 18-wk acclimation to summer or winter photoperiod conditions, the acute toxic responses of the fish groups to pulpmill effluent were compared. Effluent was a single grab sample of untreated bleached kraft whole mill effluent (BKME) from a British Columbia coastal mill. Its characteristics included pH 5.4, sodium 218 $mg \cdot L^{-1}$, color 1830 APHA, total organic carbon 270 $mg \cdot L^{-1}$, and BOD_5 192 $mg \cdot L^{-1}$. This sample was transported in sealed opaque polyethylene containers and stored at 5 ± 1°C until used (within 1 wk of collection). Just before each test, effluent portions required were adjusted to pH 6.5 (that of the dilution water) with dilute NaOH or H_2SO_4. The effluent was then heated to 11.5°C or 20°C (sealed-jar test only) and saturated with oxygen. Oxygen saturation was achieved by bubbling cylinder O_2 through air stones for 2–3 min.

Fish loading densities were 0.5 $g \cdot L^{-1}$ in all bioassays except the sealed-jar test where loadings were held at 5 $g \cdot L^{-1}$. The 96-h LC50 values for each photoperiod group were determined immediately before the sublethal tests. Test solutions for these lethal bioassays were renewed (90%) at 24-h intervals by siphoning and batch replacement. For the blood-sugar test, groups of 12 fish were held undisturbed in freshwater for 48 h after transfer to test tanks, followed by a 4-h exposure to effluent strengths equivalent to 0.0 (control), 0.2 or 0.8 times their respective 96-h LC50 value. Immediately thereafter all fish in each tank were netted in rapid succession and their blood collected from the severed caudal peduncle for subsequent plasma glucose analyses (Dubowski 1962). For the sealed-jar test, fish from each photoperiod group were placed in individual glass jars containing effluent or water (10

replicate jars per treatment). Jars were filled, sealed, and the residual oxygen level measured at the time of death of each fish using a portable oxygen analyzer probe and attached stirrer inserted into the jar. For the temperature tolerance test, groups of 10 fish from each stock tank were placed in a series of test tanks (10 fish per tank) containing water or an effluent concentration at 11.5°C. The temperature of each test solution was increased progressively at 1°C·h^{-1} until all fish died (within 18 h). The temperature at the time of death of each fish was recorded. Concentrations of effluent tested in the sealed-jar bioassay were equivalent to 0.0, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 2.5, and 3.0 times the LC50 value for each fish group; those tested in the temperature tolerance bioassay were 0.0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 1.0 LC50.

The LC50 bioassays and acclimation period for the blood-sugar test were conducted under the photoperiod conditions to which the fish were acclimated. For each toxicity test and each fish group, all BKME exposures were initiated 4 h after the onset of the respective light cycle. Lighting was continuous during the 4.5-h sealed-jar test, 18-h temperature tolerance test, and the 4-h blood-sugar test. At the time of the blood-sugar test 16 fish were sampled directly from each stock tank, 4 h after the onset of lights; blood and tissues were collected for plasma glucose analyses and liver and muscle glycogen determinations (Montgomery 1957). Other test conditions and procedures have been described previously (McLeay and Howard 1977). For the lethal bioassays, temperature tolerance, and sealed-jar tests, the LC50 and EC50 (median effective concentration) values and their 95% confidence intervals were calculated by log-probit analyses according to Finney (1971) and APHA et al. (1975). Differences between the LC50 or EC50 values determined for each photoperiod group were tested for significance according to Sprague and Fogels (1977). Plasma glucose values for fish exposed to 0.2 or 0.8 LC50 effluent were tested for significance of difference from their corresponding control values by analysis of variance and the *t*-test for differences among several means (Bruning and Kintz 1968).

Results and discussion — Lengths, weights, and condition factors as determined for groups of 25 fish sampled randomly from each tank at weekly intervals throughout the 18-wk acclimation were similar ($P > 0.05$, Student's *t*-test) for each comparison. Condition factors were 1.0 or greater and fish in each tank appeared healthy with negligible mortalities. At the time of the toxicity tests the fish acclimated to 16L:8D measured 8.5 ± 1.1 cm (mean ± SD) and 7.3 ± 2.7 g; and those acclimated to 8L:16D were 8.1 ± 1.0 cm and 6.2 ± 2.4 g.

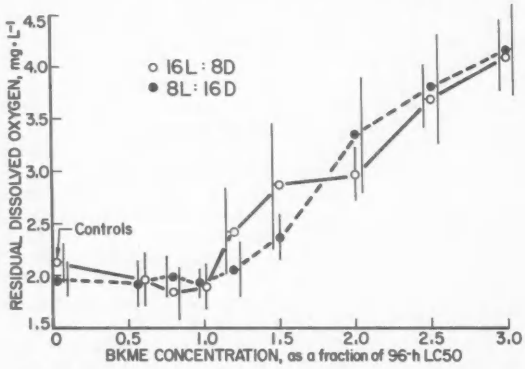

FIG. 1. Response of the sealed-jar bioassay to BKME for underyearling rainbow trout acclimated to summer (16L:8D) or winter (8L:16D) photoperiods. Points represent mean values for 10 fish and bars indicate 95% confidence intervals.

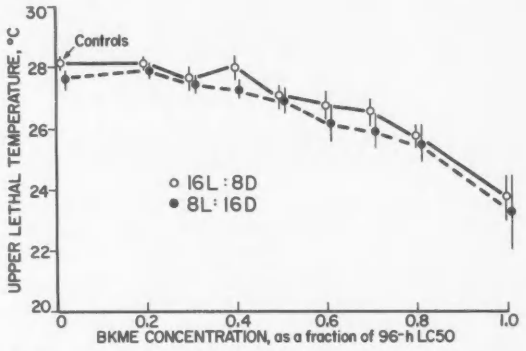

FIG. 2. Response of the temperature tolerance test to BKME for underyearling rainbow trout acclimated to summer (16L:8D) or winter (8L:16D) photoperiods. Points represent mean values for 10 fish and bars indicate 95% confidence intervals.

The acute lethal tolerance to BKME for the two fish groups was nearly identical and unaffected by photoperiod ($P > 0.05$). The 96-h LC50 values were 17.0% by volume (16.1, 17.9) and 17.4% (16.7, 18.2) for fish acclimated to summer or winter photoperiods, respectively (95% confidence interval in parentheses). The response of both fish groups to pulpmill effluent in the sealed-jar test was unaffected by day length (Fig. 1). This test was completed for both photoperiod groups within 4.5 h. Median effective concentrations (EC50) were 1.3 LC50 (1.1, 1.6) and 1.5 LC50 (1.3, 1.6) for 16L:8D and 8L:16D groups, respectively ($P > 0.05$). The EC50 values for the temperature tolerance test also did not differ significantly due to acclimation photoperiod (0.38 LC50 [0.32, 0.45] and 0.39 LC50 [0.33, 0.45] for 16L:8D and 8L:16D, respectively). However, mean upper lethal temperatures for the control and effluent-exposed fish acclimated to a summer photoperiod were consistently higher (but $P > 0.05$) than those for fish acclimated to a winter photoperiod (Fig. 2). Insufficient data were available to calculate EC50 values for the blood-sugar stress bioassay, although values for both fish groups were <0.2 LC50 since significant responses ($P < 0.01$) were obtained at this and the higher effluent strength tested (Table 1). Mean plasma glucose values for the control and effluent-exposed fish acclimated to 16L:8D were somewhat higher than respective values for fish acclimated to

TABLE 1. Carbohydrate energy reserves and the response of the blood-sugar stress bioassay to BKME for underyearling rainbow trout acclimated to summer (16L:8D) or winter (8L:16D) photoperiod.

Effluent concn (portion of 96-h LC50)	Liver glycogen (mg·g wet wt^{-1})		Muscle glycogen (mg·g wet wt^{-1})		Plasma glucose (mg %)	
	16L:8D	8L:16D	16L:8D	8L:16D	16L:8D	8L:16D
stock sample	43.2[a]	47.6	2.2	2.6	67.6	64.5
	(17.0)	(26.2)	(1.2)	(2.0)	(5.6)	(5.1)
0.0	—[b]	—	—	—	77.0	67.7
(control)	—	—	—	—	(18.0)	(14.1)
0.2	—	—	—	—	114.7[c]	94.7[c]
	—	—	—	—	(27.8)	(13.8)
0.8	—	—	—	—	124.8[c]	101.8[c]
	—	—	—	—	(39.0)	(16.1)

[a]Mean value (n = 16 for stock, 12 for 0.0–0.8 LC50 BKME) with SD in parentheses.
[b]Not determined.
[c]$P < 0.01$ that difference between effluent-exposed fish and the respective control fish is due to chance.

8L:16D. Plasma glucose values and levels of glycogen reserves for the stock samples were unaffected ($P > 0.05$, Student's t-test) by photoperiod (Table 1).

Recent evidence suggests that in fish, as in mammals, circadian variations in tolerance to toxicants exist that are susceptible to seasonal shifts in photoperiod (Reinberg and Halberg 1971; Moore Ede 1973; Spieler et al. 1977). In the present study the initiation of all effluent exposures at a fixed (4-h) interval after the onset of lights may have offset such effects. Seasonal variations in the lethal tolerance of salmonid fish have been reported even where temperature was held constant (Zitko and Carson 1977); however, such changes may be attributed to developmental changes. As with the present tests, the acute lethal tolerance (96-h LC50 values) to BKME for juvenile rainbow trout acclimated for 6 mo to constant temperature and either a fixed summer (16L:8D) or a natural (photocell) photoperiod (Gordon and McLeay 1978) was unaffected by photoperiod (unpublished finding).

The relative sensitivities of response found here with these acute toxicity tests (blood-sugar test > temperature tolerance test > LC50 ≥ sealed-jar test) are similar to results for other comparisons with coho salmon or rainbow trout exposed to BKME (McLeay and Howard 1977; unpublished data). Although seasonal changes in oxygen uptake rates that may be attributable to photoperiod have been reported for fish maintained under constant temperatures (Beamish 1964; Moore 1976), present data indicate that the residual oxygen levels for fish in sealed-jar tests are unaffected by photoperiod. The observation of a slight but consistent increase in upper lethal temperature tolerance for control or effluent-exposed fish acclimated to the longer day length (Fig. 2) has been reported for other species in similar studies without the presence of toxicants (Hoar 1956; Terpin et al. 1976). This minor alteration in values due to photoperiod does not affect the sensitivity of response of the temperature tolerance test to pulpmill effluent. Similarly in the blood-sugar stress bioassay the slight but consistent increase in mean plasma glucose levels for control or effluent-exposed fish acclimated to the longer day length did not alter the test's sensitivity to pulpmill effluent (Table 1). The absence of effect of photoperiod on glycogen energy reserves for this species has been found previously (Gordon and McLeay 1978).

The present findings indicate that the acute toxic responses examined here for rainbow trout exposed to kraft pulpmill effluent are not affected appreciably by the day length to which the fish are acclimated. It remains to be determined whether, under controlled laboratory conditions, equivalent sensitivities of response can be obtained with these and other acute toxicity tests throughout the year. Seasonal equivalence will likely require standardization of temperature and developmental stage; standardization of the photoperiodic acclimation appears to be less important than we had anticipated.

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Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

2505. Wu, R. S. S., and C. D. Levings. 1978. An energy budget for individual barnacles (*Balanus glandula*). Mar. Biol. 45: 225–235. (3)

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ANNOUNCEMENTS/AVIS

Atlantic Fisheries Technological Conference

The 23rd annual conference will be held November 5–8, 1978, at the Williamsburg Lodge, Williamsburg, Virginia. The first conference in 1957 also took place in Williamsburg.

The conference will provide a forum where fisheries technologists can discuss research objectives and methods, exchange research concepts and hypotheses, and present informal reports on researches completed or in progress. From tentative beginnings, the Conference has grown to international stature, with past sessions held in most eastern states and in Canada. Topics proposed for the contributed papers include energy, underutilized species, economics, seafood composition, quality, and nutrition.

Further information on hotel reservations and registration for the conference can be obtained from

Conférence technologique des pêches de l'Atlantique

La 23e conférence annuelle se tiendra à la Williamsburg Lodge, Williamsburg, Virginie, du 5 au 8 novembre 1978. La première conférence, en 1957, avait également eu lieu à Williamsburg.

La conférence fournira aux technologues des pêches l'occasion de discuter des objectifs et des méthodes de recherche, d'échanger des concepts et des hypothèses, et de présenter sans formalités des rapports sur des recherches terminées ou en cours. Après de modestes débuts, la conférence a acquis une réputation internationale et s'est réunie dans le passé dans la plupart des Etats de l'est des Etats-Unis et au Canada. Les sujets proposés pour les travaux qui y seront présentés comprennent l'énergie, les espèces sous-utilisées, l'économique, la composition des fruits de mer, la qualité et la nutrition.

Pour plus de renseignements sur les réservations d'hôtels et l'enregistrement à la conférence, on est prié de s'adresser à

John Long
Secretary, 23rd AFTC
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Virginia Polytechnic Institute and State University
Blacksburg, Va., 24061
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Telephone (703) 951-6962

BULLETIN 194F

Ressources marines vivantes de Terre-Neuve et du Labrador: Situation actuelle et possibilités d'exploitation

A. T. PINHORN, ÉD.

Le Bulletin 154 de l'Office des recherches sur les pêcheries du Canada constituait un résumé des connaissances acquises avant 1964 sur les ressources de la pêche à Terre-Neuve et au Labrador. Le présent bulletin résume les connaissances additionnelles dont nous nous sommes enrichis depuis. Il traite de plus des fondements scientifiques de la réglementation et de la gestion dans la zone internationale de la région de Terre-Neuve et du Labrador assurées par la Commission internationale des pêcheries de l'Atlantique nord-ouest (CIPANO).

Ce bulletin traite de toutes les espèces de la zone concernée qui ont ou pourraient avoir une valeur commerciale. Chaque article est plus ou moins détaillé suivant que l'espèce en cause est plus ou moins connue ou qu'elle a été ou non traitée antérieurement. L'étude procède par espèces et même, quand c'est possible, par stocks d'une même espèce. Les renseignements concernent la répartition, l'abondance, la biologie générale et la pêche de l'espèce; ils sont plus étoffés lorsqu'il s'agit d'un premier exposé. On trouvera également des détails sur les mesures de gestion s'appliquant aux nombreuses espèces qui ont fait l'objet de divers règlements avant et après 1970. Enfin, un aperçu du potentiel d'accroissement des prises est donné pour chaque espèce.

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